

# Chronic 1,25-Dihydroxyvitamin D<sub>3</sub> Administration in the Rat Reduces the Serum Concentration of 25-Hydroxyvitamin D by Increasing Metabolic Clearance Rate

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## Abstract

Administration of 1,25-dihydroxyvitamin D<sub>3</sub> [1,25(OH)<sub>2</sub>D<sub>3</sub>] can lower the serum concentration of 25-hydroxyvitamin (25-OH-D). To determine if 1,25(OH)<sub>2</sub>D<sub>3</sub> lowers serum 25-OH-D by increasing clearance or reducing production, we directly measured the metabolic clearance rate (MCR) of 25-OH-D in rats chronically infused with 1,25(OH)<sub>2</sub>D<sub>3</sub>. Chronic 1,25(OH)<sub>2</sub>D<sub>3</sub> administration (0 to 75 pmol/d) reduced, in a time- and dose-dependent fashion, the serum concentrations of 25-OH-D<sub>3</sub> and 24,25(OH)<sub>2</sub>D<sub>3</sub> from 18±2 to 9±1 ng/ml and from 4.8±0.7 to 1.3±0.3 ng/ml, respectively, and increased sevenfold the in vitro conversion of 25-OH-D to 24,25(OH)<sub>2</sub>D<sub>3</sub> by kidney homogenates. The reduction in serum 25-OH-D<sub>3</sub> was completely accounted for by an increase in MCR. No change in production occurred. The influence of 1,25(OH)<sub>2</sub>D<sub>3</sub> on serum 25-OH-D<sub>3</sub> and 24,25(OH)<sub>2</sub>D<sub>3</sub> was shown not to be dependent on induction of hypercalcemia. These data suggest that chronic 1,25(OH)<sub>2</sub>D<sub>3</sub> administration lowers serum 25-OH-D by increasing the metabolic clearance of 25-OH-D<sub>3</sub> and not by decreasing its production.

## Introduction

25-Hydroxyvitamin D (25-OH-D)<sup>1</sup> is produced in the liver by hydroxylation of vitamin D. 25-Hydroxyvitamin D in turn acts as substrate for production of 1,25-dihydroxyvitamin D [1,25(OH)<sub>2</sub>D], the most biologically active form of the vitamin (1, 2).

Most evidence suggests that the serum concentration of 25-OH-D is only loosely regulated and under most circumstances appears to be primarily dependent on the circulating concentration of vitamin D (3–6). Recent evidence, however, has been interpreted to suggest that 1,25(OH)<sub>2</sub>D may feed back to inhibit synthesis of 25-OH-D (7–13). For example, administration of 1,25(OH)<sub>2</sub>D has been shown to reduce the response of serum 25-OH-D<sub>3</sub> to challenge with vitamin D<sub>3</sub> (11), and osteoporotic patients given 1,25(OH)<sub>2</sub>D<sub>3</sub> have been shown to have lower serum concentrations of 25-OH-D after 1,25(OH)<sub>2</sub>D<sub>3</sub> therapy (8).

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1. Abbreviations used in this paper: 25-OH-D, 25-hydroxyvitamin D; 1,25(OH)<sub>2</sub>D, 1,25-dihydroxyvitamin D; MCR, metabolic clearance rate; PR, production rate; PTH, parathyroid hormone.

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Numerous other clinical observations support the fact that 1,25(OH)<sub>2</sub>D<sub>3</sub> can reduce circulating 25-OH-D concentrations (7, 9, 12). Furthermore, it has been shown in vitro that 1,25(OH)<sub>2</sub>D<sub>3</sub> at physiological concentrations can reduce accumulation of [<sup>3</sup>H]25-OH-D<sub>3</sub> produced from <sup>3</sup>H-vitamin D<sub>3</sub> in liver homogenates and perfused livers from rachitic rats (10). Taken collectively, these data support the hypothesis that 1,25(OH)<sub>2</sub>D can inhibit 25-OH-D synthesis. However, the data can equally well be explained by a 1,25(OH)<sub>2</sub>D-induced increase in metabolic turnover or catabolism of 25-OH-D. To determine if administration of 1,25(OH)<sub>2</sub>D in vivo lowers serum 25-OH-D by reducing production or increasing metabolic clearance, we infused rats with 1,25(OH)<sub>2</sub>D<sub>3</sub> for 12 d and directly measured the metabolic clearance rate (MCR) of 25-OH-D<sub>3</sub>. We also examined the influence of chronic 1,25(OH)<sub>2</sub>D<sub>3</sub> administration on the serum concentration of 24,25-dihydroxyvitamin D [24,25(OH)<sub>2</sub>D<sub>3</sub>], a major metabolic product of 25-OH-D and on the in vitro metabolism of 25-OH-D<sub>3</sub>, 24,25(OH)<sub>2</sub>D<sub>3</sub>, and 1,25(OH)<sub>2</sub>D<sub>3</sub> by liver and kidney homogenates. The results indicate that chronic administration of 1,25(OH)<sub>2</sub>D<sub>3</sub> can lower the serum concentrations of both 25-OH-D<sub>3</sub> and 24,25(OH)<sub>2</sub>D<sub>3</sub>, but, unlike previous in vitro reports, suggest that 1,25(OH)<sub>2</sub>D<sub>3</sub> lowers serum 25-OH-D<sub>3</sub> by increasing its metabolic clearance and not by decreasing its production.

## Methods

**Animal protocols.** Male Sprague-Dawley rats (Hilltop Laboratories, Scottsdale, PA) weighing 100–125 g were fed ad lib. either standard rat chow (Wayne Lab Blox, Allied Mills, Inc., Chicago, IL, F-6) containing 4.41 U vitamin D<sub>3</sub>/g of food, 1.2% Ca and 0.99% P, or one of three synthetic vitamin D-deficient diets supplemented with vitamin D<sub>3</sub> (100 U vitamin D<sub>3</sub>/d) and containing either 0.4, 1.2, or 2.4% Ca, and 1.2% P, respectively (14). All animals were maintained on their respective diets for at least 10 d before experimentation and at the time of killing were 55 d of age in each experiment.

To examine the effect of chronic 1,25(OH)<sub>2</sub>D<sub>3</sub> administration on the serum concentrations of 1,25(OH)<sub>2</sub>D<sub>3</sub>, 25-OH-D<sub>3</sub>, and 24,25(OH)<sub>2</sub>D<sub>3</sub>, Alza osmotic minipumps (model 2002, Alza Corp., Palo Alto, CA) were implanted subcutaneously on the back and the animals were continuously infused with either vehicle (1.25% ethanol in propylene glycol) or chromatographically purified 1,25(OH)<sub>2</sub>D<sub>3</sub> (a gift from Dr. M. Uskokovic, Hoffmann-LaRoche, Nutley, NJ) at a constant rate of 25, 50 or 75 pmol/d (12 µl/d) for 3, 7, or 12 d. At the end of the infusion period, blood was collected from the dorsal aorta while the animals were under light ether anesthesia and the serum analyzed for calcium, inorganic phosphate, and the vitamin D metabolites.

The MCR of 25-OH-D<sub>3</sub> was measured using the constant infusion method (15, 16). Approximately 0.5 µCi of chromatographically purified 25-OH-(26,27(n)-<sup>3</sup>H)D<sub>3</sub> (158 Ci/mmol) was solubilized in propylene glycol containing 1.25% ethanol and continuously infused subcutaneously using Alza osmotic minipumps at a rate of between 30 and 60 dpm/min (8.33 nl/min) for up to 13 d. This rate of hormone infusion is equivalent to

0.035–0.07 pg/min or 0.008–0.016% of the endogenous production rate. For all routine measurements of MCR, animals were infused for 12 d. At the end of the infusion period, animals were bled from the dorsal aorta.

Accurate measurement of the MCR using the constant infusion technique requires that the serum pool of [ $^3\text{H}$ ]25-OH-D<sub>3</sub>, at the time of measuring the MCR, be in equilibrium with all other pools of [ $^3\text{H}$ ]25-OH-D<sub>3</sub> within the animal. To determine if the serum pool of [ $^3\text{H}$ ]25-OH-D<sub>3</sub> was at equilibrium (i.e., the concentration of [ $^3\text{H}$ ]25-OH-D<sub>3</sub> in the serum was constant) at the time of measuring the MCR, in preliminary experiments we measured the serum concentration of [ $^3\text{H}$ ]25-OH-D<sub>3</sub> at 2, 6, 9, and 13 d after beginning the infusion. The serum concentrations of [ $^3\text{H}$ ]25-OH-D<sub>3</sub> ( $n = 4$ ) at these time points were  $1,300 \pm 116$ ,  $3,405 \pm 147$ ,  $3,508 \pm 556$ , and  $3,795 \pm 310$  dpm/ml, respectively. No significant differences (Tukey test) were seen in the concentrations at days 6, 9, and 13 indicating that the serum pool of [ $^3\text{H}$ ]25-OH-D<sub>3</sub> was in fact in equilibrium at the time of measuring the MCR (i.e., day 12).

**In vitro metabolism studies.** To determine the effect of chronic 1,25(OH)<sub>2</sub>D<sub>3</sub> administration on the in vitro metabolism of 25-OH-D<sub>3</sub>, 24,25(OH)<sub>2</sub>D<sub>3</sub>, and 1,25(OH)<sub>2</sub>D<sub>3</sub> by liver and kidney, animals were infused with either vehicle alone or 1,25(OH)<sub>2</sub>D<sub>3</sub> at the rate of 75 pmol/d for 12 d and then sacrificed. After killing, livers and kidneys were perfused in vivo with 10 ml ice-cold homogenizing buffer, excised, passed through a Krebs tissue press, and homogenized using a teflon pestle in 2 vol of the following buffer: 100 mM KCl, 10 mM Tris-HCl, 10 mM NaH<sub>2</sub>PO<sub>4</sub>, 10 mM Na-pyruvate, 4 mM MgSO<sub>4</sub> and 10 mM EGTA with CaCl<sub>2</sub> added to a free calcium concentration of 0.01 mM at pH 6.8. 1-ml aliquots of the homogenized tissue were combined with  $\sim 0.1 \mu\text{Ci}$  of either [ $^3\text{H}$ ]25-OH-D<sub>3</sub> (85 Ci/mmol), [ $^3\text{H}$ ]24,25(OH)<sub>2</sub>D<sub>3</sub> (64 Ci/mmol) or [ $^3\text{H}$ ]1,25(OH)<sub>2</sub>D<sub>3</sub> (158 Ci/mmol) in 10  $\mu\text{l}$  of ethanol, gassed with oxygen, and incubated (in duplicate) in a shaking water bath at 37°C for 2 h. The incubations were stopped by adding 3 ml of a 2:1 mixture of methanol:chloroform, the samples shaken vigorously and phase separation accomplished by adding an additional 1 ml of chloroform and 0.8 ml H<sub>2</sub>O. After centrifugation at 3,000 rpm for 5 min in a clinical bench top centrifuge, the lower (chloroform) phase was removed and the upper aqueous phase reextracted twice with 1 ml chloroform. The chloroform extracts were combined, dried under nitrogen and resublimized in 150  $\mu\text{l}$  isopropanol/hexane (10:90) in preparation for high performance liquid chromatography. The chromatographic system consisted of a Zorbax-Sil column (Dupont Instruments, Wilmington, DE) and a solvent system of isopropanol/hexane run in a concave gradient mode (setting 9, Waters 660 programmer, Waters Associates, Milford, MA) from 3:97 to 10:90 at a flow rate of 2 ml/min. Tritium in the column effluent was monitored continuously using a Flo-One radioactive flow counter (Radiomatics Instrument and Chemical Co., Tampa, FL). Because the tissue pools of endogenous 25-OH-D, 24,25(OH)<sub>2</sub>D, and 1,25(OH)<sub>2</sub>D can theoretically influence the rate of metabolic conversion of [ $^3\text{H}$ ]25-OH-D, [ $^3\text{H}$ ]24,25(OH)<sub>2</sub>D and [ $^3\text{H}$ ]1,25(OH)<sub>2</sub>D to other metabolites, we also measured the kidney and liver homogenate concentrations of endogenous 25-OH-D, 24,25(OH)<sub>2</sub>D, and 1,25(OH)<sub>2</sub>D.

**Laboratory methods.** The serum, kidney, and liver concentrations of 1,25(OH)<sub>2</sub>D<sub>3</sub>, 25-OH-D<sub>3</sub>, and 24,25(OH)<sub>2</sub>D<sub>3</sub> were measured in the following manner. 2,000–4,000 dpm each of chromatographically purified 1,25(OH)<sub>2</sub> [26,27( $n$ )- $^3\text{H}$ ]D<sub>3</sub> (158 Ci/mmol), 25-OH[26,27( $n$ )- $^3\text{H}$ ]D<sub>3</sub> (158 Ci/mmol), and 24,25(OH) [23,24( $n$ )- $^3\text{H}$ ]D<sub>3</sub> (66 Ci/mmol) (Amersham Corp., Arlington Heights, IL) in 20  $\mu\text{l}$  of ethanol were added to 2 ml of serum and the serum was allowed to sit at room temperature for 10 min. Samples were then extracted twice with 3 vol of peroxide-free diethylether (J. T. Baker Chemical Corp., Phillipsburg, NJ) followed by extraction with 4 vol of dichloromethane/methanol (3:1). After mixing for 5 min, 1 additional vol of methanol was added, the samples were shaken for an additional 2 min and the methanol-aqueous layer was removed by aspiration. The remaining solution was washed with 0.1 M sodium phosphate buffer (pH 10.5) twice. The remaining organic phase was combined with the ether extracts, dried under nitrogen, applied to a Sephadex LH-20 column (0.6  $\times$  15 cm) and eluted in hexane/chloroform/methanol (90:10:10). The 25-OH-D<sub>3</sub> fraction from LH-20 chromatography was

applied to a Waters Radial Compression Separation System (Waters Associates) using an 8-mm silica radial compression column (10  $\mu\text{m}$  particle size), a solvent system of isopropanol/hexane (4:96) and a flow rate of 3 ml/min. 25-OH-D<sub>3</sub> was quantitated using a competitive protein binding assay based on vitamin D-deficient rat serum as previously described (17). Inter- and intraassay coefficients of variation for this assay are 16 and 10%, respectively.

The dihydroxyvitamin D<sub>3</sub> fraction from LH-20 chromatography was applied to two  $\mu\text{Porosil}$  columns in series and eluted with a solvent system of isopropanol/hexane (10:90) at a flow rate of 2 ml/min. The 1,25(OH)<sub>2</sub>D<sub>3</sub> fraction from this chromatography was quantitated with the chick intestinal cytosol receptor assay as described by Shepard et al. (18). Inter- and intraassay coefficients of variation for this assay are 11 and 9%, respectively. The 24,25(OH)<sub>2</sub>D<sub>3</sub> fraction from the two  $\mu\text{Porosil}$  columns was further chromatographed on a Zorbax-Sil column with a solvent system of isopropanol/dichloromethane (2:98) and a flow rate of 2 ml/min (19). 24,25-Dihydroxyvitamin D<sub>3</sub> was quantitated using a competitive protein binding assay based on vitamin D deficient rat serum as previously described (16). Inter- and intraassay coefficients of variation for this assay are 20 and 12%, respectively.

For measurement of the MCR, the serum concentration of [ $^3\text{H}$ ]25-OH-D<sub>3</sub> was measured in the following manner. Approximately 1.2  $\mu\text{g}$  of chromatographically purified 25-OH-D<sub>3</sub> in 20  $\mu\text{l}$  of ethanol was added to each 2-ml serum sample to determine recovery. Samples were extracted and chromatographed using a C-18 Sep-Pak (Waters Associates) as described by Reinhardt et al. (12) followed by chromatography on a silica Sep-Pak (Waters Associates). Samples were applied to the silica Sep-Paks in isopropanol/hexane (4:96) and eluted with 8 ml of the same solvent. The 25-OH-D fraction was then chromatographed using an 8-mm silica radial compression column (10  $\mu\text{m}$  particle size), a solvent system of isopropanol/hexane (3:97) and a flow rate of 3.5 ml/min, followed by further high performance liquid chromatography using a  $\mu\text{Bondapak}$  C-18 column, a solvent system of water/methanol (22:78) and a flow rate of 2.0 ml/min. The 25-OH-D<sub>3</sub> fractions from the  $\mu\text{Bondapak}$  C-18 column were dried under nitrogen and the amount of  $^3\text{H}$  determined by scintillation counting. Calculation of recovery of 25-OH-D<sub>3</sub> added to the serum was accomplished by comparison of the ultraviolet light absorbance maximum at 254 nm of the sample to that of 25-OH-D<sub>3</sub> standards. Recovery of 25-OH-D<sub>3</sub> averaged 51%. The intraassay coefficient of variation for measurement of [ $^3\text{H}$ ]25-OH-D<sub>3</sub> in serum is 5.9% ( $n = 7$ ).

To insure that the chromatographic procedures used to purify [ $^3\text{H}$ ]25-OH-D<sub>3</sub> from serum were adequate (i.e., that the tritium label accurately reflected authentic [ $^3\text{H}$ ]25-OH-D<sub>3</sub> and not other substances that may have become labeled during the course of the infusion) we calculated the serum concentration of [ $^3\text{H}$ ]25-OH-D<sub>3</sub> after each step in a series of chromatographic procedures designed to sequentially improve purity of the sample. In these experiments, 1.2  $\mu\text{g}$  of nonlabeled 25-OH-D<sub>3</sub> in 20  $\mu\text{l}$  of ethanol was added to each sample of a group ( $n = 10$ ) of serum samples (2.0 ml each) drawn from a pool of serum collected from four rats that had been infused with [ $^3\text{H}$ ]25-OH-D<sub>3</sub> for 12 d. The serum samples were extracted and chromatographed as described above with the exception that after the reverse-phase chromatography step (i.e., the  $\mu\text{Bondapak}$  column) the 25-OH-D<sub>3</sub> fractions were rechromatographed using a Zorbax-Sil column, a solvent system of isopropanol/dichloromethane (2:98) and a flow rate of 2 ml/min. After each chromatographic step (i.e., after the first straight phase, after the reverse phase, and after the second straight phase) a fraction of the sample was removed, the  $^3\text{H}$  in the fraction quantitated, and from the recovery at that step, the concentration of [ $^3\text{H}$ ]25-OH-D<sub>3</sub> calculated. The serum concentrations of [ $^3\text{H}$ ]25-OH-D<sub>3</sub> calculated after each stage of chromatography were  $3,072 \pm 237$ ,  $3,146 \pm 170$  and  $3,515 \pm 198$  dpm/ml, respectively. No significant differences (Tukey test) in the concentrations were seen, suggesting that the first straight phase high performance liquid chromatographic system was adequate to remove any  $^3\text{H}$ -labeled contaminants from the [ $^3\text{H}$ ]25-OH-D<sub>3</sub> fraction. To insure a margin of safety, however, we choose to use the straight phase followed by reverse-phase high performance liquid chromatographic system for all routine analyses.

Serum calcium was measured using atomic absorption spectroscopy after dilution (1/50) of serum with  $\text{LaCl}_3$ . Serum inorganic phosphate ( $\text{P}_i$ ) was measured using the method of LeBel et al. (20).

**Calculations.** The MCR of 25-OH- $\text{D}_3$ , defined as the volume of blood cleared completely and irreversibly of 25-OH- $\text{D}_3$  per unit time (16), is assumed to be equal to that of [ $^3\text{H}$ ]25-OH- $\text{D}_3$ . At infusion equilibrium, the MCR is calculated according to the relationship:  $\text{MCR (ml/min)} = \text{rate of infusion of } [^3\text{H}]25\text{-OH-}\text{D}_3 \text{ (dpm/min)}/\text{serum concentration of } [^3\text{H}]25\text{-OH-}\text{D}_3 \text{ (dpm/ml)}$ . The production rate (PR) of 25-OH- $\text{D}_3$  is calculated according to the relationship:  $\text{PR (pg/min)} = \text{MCR (ml/min)} \times \text{serum concentration of 25-OH-}\text{D}_3 \text{ (pg/ml)}$ .

Data are presented as mean $\pm$ SE. Statistical analysis was performed using Student's *t* test or, where appropriate, one-way analysis of variance and Dunnett's test, or two-way analysis of variance and the Tukey multiple comparison test.

## Results

Chronic administration of 1,25(OH) $_2\text{D}_3$  at a dose of 25 pmol/d significantly increased serum 1,25(OH) $_2\text{D}_3$  by 19–24 pg/ml ( $P < 0.05$ ) (Table I). Further increases in dosage produced a nearly linear increase in the serum concentration of 1,25(OH) $_2\text{D}_3$  ( $r = 0.76$ ,  $P < 0.001$ ). The duration of 1,25(OH) $_2\text{D}_3$  infusion (from 3–12 d) did not influence the serum 1,25(OH) $_2\text{D}_3$  levels achieved.

After 3 d of 1,25(OH) $_2\text{D}_3$  administration, no changes in serum 25-OH- $\text{D}_3$  were seen except at the highest dose where a small decrement was observed ( $21 \pm 3$  ng/ml in control and  $16 \pm 2$  ng/ml in 1,25(OH) $_2\text{D}_3$  infused) (Table I). However, after 7 and 12 d, serum 25-OH- $\text{D}_3$  was significantly reduced ( $P < 0.001$ ) at all three doses of 1,25(OH) $_2\text{D}_3$ . After 12 d of 1,25(OH) $_2\text{D}_3$  infusion, at a dose of 25 pmol/d, serum 25-OH- $\text{D}_3$  fell from a control level of  $18 \pm 2$  ng/ml to  $12 \pm 1$  ng/ml ( $P < 0.001$ ). Increasing the 1,25(OH) $_2\text{D}_3$  dose to 50 pmol/d further reduced serum 25-OH- $\text{D}_3$  to  $9 \pm 1$  ng/ml. However, as the 1,25(OH) $_2\text{D}_3$  dose was increased to 75 pmol/d, no further reduction in serum 25-OH-

$\text{D}_3$  occurred. The relationship between the serum concentrations of 1,25(OH) $_2\text{D}_3$  and 25-OH- $\text{D}_3$  is shown in Fig. 1. As the serum concentration of 1,25(OH) $_2\text{D}_3$  was increased to  $\sim 150$  pg/ml, there was a more or less linear decrease in the serum level of 25-OH- $\text{D}_3$ . However, for higher serum concentrations of 1,25(OH) $_2\text{D}_3$  (up to 225 pg/ml), no further reduction in serum 25-OH- $\text{D}_3$  was observed.

After 7 and 12 d of constant 1,25(OH) $_2\text{D}_3$  infusion the serum concentration of 24,25(OH) $_2\text{D}_3$  was also significantly reduced ( $P < 0.001$ ) (Table I). However, unlike the serum concentration of 25-OH- $\text{D}_3$ , a higher dose of 1,25(OH) $_2\text{D}_3$  (viz. 50 pmol/d) was required to elicit a fall in serum 24,25(OH) $_2\text{D}_3$ . After 12 d of 1,25(OH) $_2\text{D}_3$  infusion, serum 24,25(OH) $_2\text{D}_3$  was virtually identical in the 0- and 25-pmol/d dose groups. At a dose of 50 pmol/d, serum 24,25(OH) $_2\text{D}_3$  fell abruptly from  $4.8 \pm 0.7$  ng/ml (0 dose) to  $1.8 \pm 0.1$  ng/ml ( $P < 0.001$ ). Increasing the 1,25(OH) $_2\text{D}_3$  dose to 75 pmol/d reduced serum 24,25(OH) $_2\text{D}_3$  to  $1.3 \pm 0.3$  ng/ml, a level not significantly different from the 50-pmol/d dose group. The relationship between the serum concentrations of 1,25(OH) $_2\text{D}_3$  and 24,25(OH) $_2\text{D}_3$  is shown in Fig. 2. As the serum concentration of 1,25(OH) $_2\text{D}_3$  was increased to  $\sim 130$  pg/ml, there was little change in circulating 24,25(OH) $_2\text{D}_3$ . However, as serum 1,25(OH) $_2\text{D}_3$  was further increased to 150 pg/ml there was an abrupt fall in circulating 24,25(OH) $_2\text{D}_3$  from roughly 4.6 to 1.4 ng/ml.

Chronic 1,25(OH) $_2\text{D}_3$  administration produced a dose-dependent increase ( $P < 0.001$ ) in the serum concentration of calcium but had little effect (with the exception of the 3-d time point) on the serum concentration of inorganic phosphate ( $\text{P}_i$ ) (Table I). At 3, 7, and 12 d after beginning infusion of 1,25(OH) $_2\text{D}_3$  serum calcium was significantly ( $P < 0.001$ ) increased in the 50- and 75-pmol/d dose groups. In the 25-pmol/d dose groups, although there was a trend for serum calcium to be increased, the difference did not reach significance ( $P = 0.250$ ).

To determine if the reduction in serum 25-OH- $\text{D}_3$  brought

Table I. Effects of Chronic 1,25(OH) $_2\text{D}_3$  Infusion on the Serum Concentrations of 1,25(OH) $_2\text{D}_3$ , 25-OH- $\text{D}_3$ , and 24,25(OH) $_2\text{D}_3$ , Ca, and  $\text{P}_i$

| Days after beginning infusion | Serum concentrations* of     |                         |                      |                          |                          |                  |
|-------------------------------|------------------------------|-------------------------|----------------------|--------------------------|--------------------------|------------------|
|                               | 1,25(OH) $_2\text{D}_3$ dose | 1,25(OH) $_2\text{D}_3$ | 25-OH- $\text{D}_3$  | 24,25(OH) $_2\text{D}_3$ | Ca                       | $\text{P}_i$     |
|                               | pmol/d                       | pg/ml                   | ng/ml                | ng/ml                    | mg/dl                    | mg/dl            |
| 3                             | 0                            | $113 \pm 15$            | $21 \pm 3$           | —                        | $10.0 \pm 0.1$           | $7.8 \pm 0.3$    |
|                               | 25                           | $132 \pm 8^\ddagger$    | $20 \pm 2$           | —                        | $10.2 \pm 0.2$           | $8.2 \pm 0.2^\S$ |
|                               | 50                           | $159 \pm 6^\ddagger$    | $20 \pm 2$           | —                        | $10.5 \pm 0.2^\parallel$ | $8.3 \pm 0.1^\S$ |
|                               | 75                           | $180 \pm 10^\ddagger$   | $16 \pm 2$           | $4.3 \pm 0.6$            | $10.8 \pm 0.3^\parallel$ | $8.7 \pm 0.2^\S$ |
| 7                             | 0                            | $103 \pm 9$             | $20 \pm 2$           | —                        | $10.4 \pm 0.2$           | $7.6 \pm 0.3$    |
|                               | 25                           | $126 \pm 4^\ddagger$    | $13 \pm 2^\parallel$ | —                        | $10.5 \pm 0.1$           | $7.7 \pm 0.1$    |
|                               | 50                           | $166 \pm 10^\ddagger$   | $11 \pm 1^\parallel$ | —                        | $11.0 \pm 0.2^\parallel$ | $8.2 \pm 0.2$    |
|                               | 75                           | $180 \pm 13^\ddagger$   | $10 \pm 1^\parallel$ | $1.2 \pm 0.1$            | $11.1 \pm 0.3^\parallel$ | $7.8 \pm 0.3$    |
| 12                            | 0                            | $100 \pm 10$            | $18 \pm 2$           | $4.8 \pm 0.7$            | $10.3 \pm 0.1$           | $7.7 \pm 0.3$    |
|                               | 25                           | $124 \pm 6^\ddagger$    | $12 \pm 1^\parallel$ | $4.6 \pm 0.4$            | $10.5 \pm 0.3$           | $7.5 \pm 0.1$    |
|                               | 50                           | $150 \pm 14^\ddagger$   | $9 \pm 1^\parallel$  | $1.8 \pm 0.1^{**}$       | $10.7 \pm 0.2^\parallel$ | $7.7 \pm 0.1$    |
|                               | 75                           | $175 \pm 10^\ddagger$   | $9 \pm 1^\parallel$  | $1.3 \pm 0.3^{**}$       | $11.2 \pm 0.1^\parallel$ | $7.5 \pm 0.2$    |

\* Mean $\pm$ SE, six rats per group.  $^\ddagger$  Significantly higher than 0 dose group at all times,  $P < 0.05$ , multivariate analysis, Tukey test.  $^\S$  Significantly higher than equivalent dose groups from other days,  $P < 0.05$ , multivariate analysis.  $^\parallel$  Significantly higher than 0 dose group,  $P < 0.001$ , multivariate analysis, Tukey test.  $^\#$  Significantly lower than 0 dose group,  $P < 0.001$ , multivariate analysis, Tukey test.  $^{**}$  Significantly lower than in 0 dose group,  $P < 0.001$ , analysis of variance, Dunnett's test.

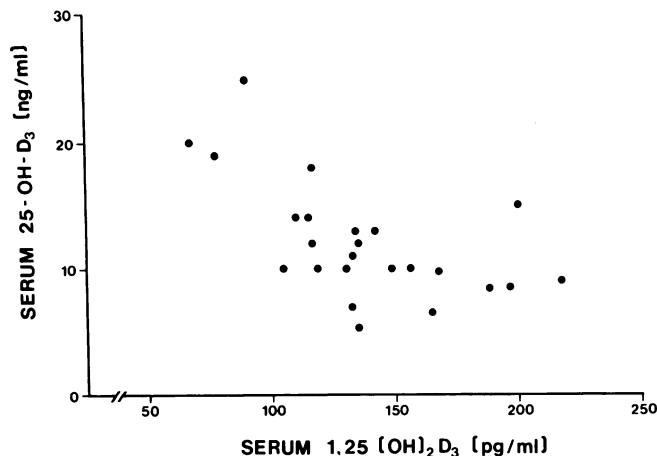


Figure 1. Relationship between the serum concentrations of 25-OH-D<sub>3</sub> and 1,25(OH)<sub>2</sub>D<sub>3</sub> in rats continuously infused (Alza osmotic minipumps) with 1,25(OH)<sub>2</sub>D<sub>3</sub> at the rate of 0, 25, 50, or 75 pmol/d for 12 d and fed a diet containing 4.41 U vitamin D<sub>3</sub>/d, 1.2% Ca and 0.99% P.

about by chronic 1,25(OH)<sub>2</sub>D<sub>3</sub> administration was a result of an increase in metabolic clearance of 25-OH-D<sub>3</sub> or a decrease in production, we directly measured the MCR of 25-OH-D<sub>3</sub> using the constant infusion method. Chronic 1,25(OH)<sub>2</sub>D<sub>3</sub> administration increased the metabolic clearance of 25-OH-D<sub>3</sub> (Table II). In animals fed a synthetic diet containing 1.2% Ca, 1.2% P, and 100 U D<sub>3</sub>/d, administration of 1,25(OH)<sub>2</sub>D<sub>3</sub> at the rate of 75 pmol/d for 12 d increased serum 1,25(OH)<sub>2</sub>D<sub>3</sub> from 140±6 to 199±6 pg/ml ( $P < 0.005$ ), decreased serum 25-OH-D<sub>3</sub> from 24±1 to 14±2 ng/ml ( $P < 0.001$ ), increased the MCR of 25-OH-D<sub>3</sub> from 17.5±0.6 to 29.7 μl/min ( $P < 0.001$ ) but did not affect the PR of 25-OH-D<sub>3</sub>.

To examine the mechanisms for the increased clearance of

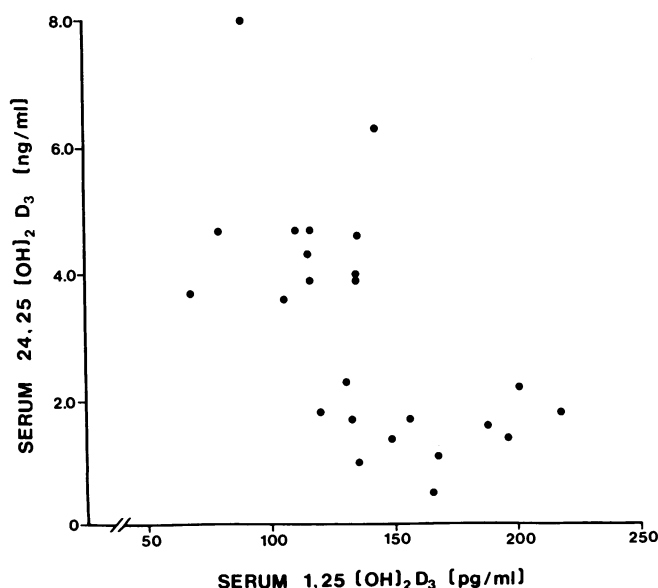


Figure 2. Relationship between the serum concentrations of 24,25(OH)<sub>2</sub>D<sub>3</sub> and 1,25(OH)<sub>2</sub>D<sub>3</sub> in rats continuously infused (Alza osmotic minipumps) with 1,25(OH)<sub>2</sub>D<sub>3</sub> at the rate of 0, 25, 50, or 75 pmol/d for 12 d and fed a diet containing 4.41 U vitamin D<sub>3</sub>/d, 1.2% Ca and 0.99% P.

Table II. Effects of Chronic 1,25(OH)<sub>2</sub>D<sub>3</sub> Infusion for 12 d on the Serum Concentration, MCR, and PR of 25-OH-D<sub>3</sub>

| 1,25(OH) <sub>2</sub> D <sub>3</sub> dose | Serum concentration*<br>of 25-OH-D <sub>3</sub> | MCR*      | PR*    |
|---|---|-----------|--------|
| pmol/d                                    | ng/ml   | ul/min    | pg/min |
| 0   | 24±1  | 17.5±0.6  | 421±29 |
| 75  | 14±2‡   | 29.7±1.8‡ | 410±30 |

\* Mean±SE, six rats per group.

‡  $P < 0.001$ , Student's *t* test, two-tailed.

25-OH-D<sub>3</sub> following 1,25(OH)<sub>2</sub>D<sub>3</sub> infusion, we evaluated the effect of chronic 1,25(OH)<sub>2</sub>D<sub>3</sub> administration in vivo on the metabolism of 25-OH-D<sub>3</sub>, 24,25(OH)<sub>2</sub>D<sub>3</sub>, and 1,25(OH)<sub>2</sub>D<sub>3</sub> in liver and kidney homogenates. Chronic 1,25(OH)<sub>2</sub>D<sub>3</sub> administration (12 d, 75 pmol/d) resulted in virtually no change in the in vitro metabolism of 25-OH-D<sub>3</sub>, 24,25(OH)<sub>2</sub>D<sub>3</sub>, and 1,25(OH)<sub>2</sub>D<sub>3</sub> by liver homogenates (Table III). On the contrary, 1,25(OH)<sub>2</sub>D<sub>3</sub> administration (12 d, 75 pmol/d) substantially increased the in vitro metabolic conversion of 25-OH-D<sub>3</sub> to 24,25(OH)<sub>2</sub>D<sub>3</sub> in renal homogenates. In preparations of renal tissue from animals infused with vehicle alone, 89.4% of the original 25-OH-D<sub>3</sub> added to the incubation media was recovered intact, while 3.1% was converted to 24,25(OH)<sub>2</sub>D<sub>3</sub>. In preparations of renal tissue from animals chronically infused with 1,25(OH)<sub>2</sub>D<sub>3</sub> for 12 d at a rate of 75 pmol/d, only 71.7% of the original 25-OH-D<sub>3</sub> added to the incubation media was recovered intact while 21.6% was converted to 24,25(OH)<sub>2</sub>D<sub>3</sub>. Catabolism of 24,25(OH)<sub>2</sub>D<sub>3</sub> in renal preparations in two of three independent experiments was similar in vehicle- and 1,25(OH)<sub>2</sub>D<sub>3</sub>-infused animals. In the third experiment, an increase in catabolism was observed. The reason for this anomaly is not clear. Catabolism of 1,25(OH)<sub>2</sub>D<sub>3</sub>, which was very rapid in control animals, was further increased in animals chronically infused with 1,25(OH)<sub>2</sub>D<sub>3</sub>. Kidney concentrations of endogenous 25-OH-D in vehicle and 1,25(OH)<sub>2</sub>D<sub>3</sub> infused animals were 0.7 ng/ml homogenate and 0.2 ng/ml homogenate (mean of duplicate determinations on pooled tissue homogenates), respectively. These concentrations of endogenous 25-OH-D are at least two orders of magnitude less than the Michaelis constant ( $K_m$ ) of the 1- and 24-hydroxylase for 25-OH-D (21). Liver concentrations of endogenous 25-OH-D in vehicle- and infused animals were 0.4 ng/ml homogenate and 0.5 ng/ml homogenate, respectively. Liver and kidney concentrations of 24,25(OH)<sub>2</sub>D and 1,25(OH)<sub>2</sub>D in vehicle- and 1,25(OH)<sub>2</sub>D<sub>3</sub>-infused animals were all ≤100 pg/ml of homogenate. These concentrations are <15% of the concentration of added substrate.

Because infusion of 1,25(OH)<sub>2</sub>D<sub>3</sub> increases the serum concentrations of both 1,25(OH)<sub>2</sub>D<sub>3</sub> and calcium, it is difficult to determine whether the increased serum concentration of 1,25(OH)<sub>2</sub>D<sub>3</sub> or the accompanying hypercalcemia or both are responsible for the decreases in serum 25-OH-D<sub>3</sub> and 24,25(OH)<sub>2</sub>D<sub>3</sub>. To differentiate among these possibilities, animals were fed a synthetic vitamin D-deficient diet supplemented with 100 U of vitamin D<sub>3</sub>/d and containing 1.2% phosphorus and either 0.4, 1.2, or 2.4% calcium in order to modulate the changes in serum calcium brought about by 1,25(OH)<sub>2</sub>D<sub>3</sub> infusion. Animals from each diet group were infused with either vehicle alone or 1,25(OH)<sub>2</sub>D<sub>3</sub> at the rate of 75 pmol/d for 12 d.

Table III. In Vitro Metabolism of 25-OH-D<sub>3</sub>, 24,25(OH)<sub>2</sub>D<sub>3</sub>, and 1,25(OH)<sub>2</sub>D<sub>3</sub> by Liver and Kidney Homogenates from Rats Chronically Infused with 1,25(OH)<sub>2</sub>D<sub>3</sub> for 12 d

| Tissue | Substrate                             | 1,25(OH) <sub>2</sub> D <sub>3</sub> dose<br>pmol/d | Percent substrate remaining or conversion to other metabolites* |                                       |                                      |        | Aqueous§ |
|--------|---------------------------------------|---|---|---------------------------------------|--------------------------------------|--------|----------|
|        |                                       |   | 25-OH-D <sub>3</sub>  | 24,25(OH) <sub>2</sub> D <sub>3</sub> | 1,25(OH) <sub>2</sub> D <sub>3</sub> | Other‡ |          |
| Liver  | 25-OH-D <sub>3</sub>                  | 0   | 89.1  | 0                                     | 0                                    | 5.0    | 2.0      |
|        |                                       | 75  | 91.2  | 0                                     | 0                                    | 3.7    | 1.5      |
|        | 24,25(OH) <sub>2</sub> D <sub>3</sub> | 0   | 0   | 67.3                                  | 0                                    | 22.7   | 4.6      |
|        |                                       | 75  | 0   | 69.3                                  | 0                                    | 26.5   | 2.2      |
|        | 1,25(OH) <sub>2</sub> D <sub>3</sub>  | 0   | 0   | 0                                     | 96.9                                 | 1.6    | 2.4      |
|        |                                       | 75  | 0   | 0                                     | 97.8                                 | .8     | 2.0      |
| Kidney | 25-OH-D <sub>3</sub>                  | 0   | 89.4  | 3.1                                   | 0.2                                  | 0      | 2.9      |
|        |                                       | 75  | 71.7  | 21.6                                  | 0.2                                  | 0      | 4.1      |
|        | 24,25(OH) <sub>2</sub> D <sub>3</sub> | 0   | 0   | 75.7                                  | 0                                    | 13.0   | 9.0      |
|        |                                       | 75  | 0   | 80.2                                  | 0                                    | 14.2   | 11.3     |
|        | 1,25(OH) <sub>2</sub> D <sub>3</sub>  | 0   | 0   | 0                                     | 6.0                                  | 5.5    | 62.5     |
|        |                                       | 75  | 0   | 0                                     | 0                                    | 0      | 86.6     |

\* Values (mean of duplicate determinations) are expressed as a percentage of the total counts incubated with the homogenates. ‡ Other unidentified metabolites. § Percent counts remaining in the aqueous phase after extraction.

Animals infused with 1,25(OH)<sub>2</sub>D<sub>3</sub> and receiving the 2.4% calcium diet were slightly smaller than animals in the other groups (Table IV). The serum concentration of calcium in vehicle-infused animals was identical (10.9±0.1 mg/dl) when dietary calcium was 1.2 and 2.4%, but was lower (10±0.1 mg/dl,  $P < 0.001$ ) when dietary calcium was reduced to 0.4%. 1,25-Dihydroxyvitamin D<sub>3</sub> infusion increased ( $P < 0.001$ ) serum calcium by 1.0 to 1.1 mg/dl when dietary calcium was 1.2 and 2.4% but did not significantly change serum calcium when dietary calcium was 0.4%. The serum concentration of phosphate decreased significantly when dietary calcium was increased ( $P < 0.25$ ) and/or when 1,25(OH)<sub>2</sub>D<sub>3</sub> was infused ( $P < 0.005$ ).

The serum concentration of 1,25(OH)<sub>2</sub>D<sub>3</sub> in vehicle-infused animals decreased from 152±7 pg/ml to 121±pg/ml ( $P < 0.001$ ) when dietary calcium was increased from 0.4% to 2.4% (Table IV). 1,25-Dihydroxyvitamin D<sub>3</sub> infusion increased ( $P < 0.005$ ) serum 1,25(OH)<sub>2</sub>D<sub>3</sub> within each of the diet groups, although the

increment in increase was substantially less in the 0.4% dietary calcium group (23 pg/ml) than in the 2.4% dietary calcium group (61 pg/ml).

The serum concentration of 25-OH-D<sub>3</sub> in vehicle infused animals was identical (24±ng/ml) when dietary calcium was 0.4 and 1.2% but was higher (36±ng/ml,  $P < 0.001$ ) when dietary calcium was increased to 2.4% (Table IV). Regardless of the concentration of 25-OH-D<sub>3</sub> in vehicle infused animals however, chronic 1,25(OH)<sub>2</sub>D<sub>3</sub> infusion reduced serum 25-OH-D<sub>3</sub> ( $P < 0.001$ ) approximately the same amount (viz. 10–14 ng/ml) in each diet group.

The serum concentration of 24,25(OH)<sub>2</sub>D<sub>3</sub> in vehicle infused animals was similar, 10.9±0.6 and 11.4±1.1 ng/ml, when dietary calcium was 1.2 and 2.4%, respectively, but was lower, 6.8±0.9 ng/ml ( $P < 0.001$ ), when dietary calcium was reduced to 0.4% (Table IV). In each dietary group, chronic 1,25(OH)<sub>2</sub>D<sub>3</sub> infusion significantly reduced serum 24,25(OH)<sub>2</sub>D<sub>3</sub> ( $P < 0.001$ ).

Table IV. Effects of Dietary Calcium and Chronic 1,25(OH)<sub>2</sub>D<sub>3</sub> Infusion for 12 d on Body Weights and the Serum Concentrations of 25-OH-D<sub>3</sub>, 24,25(OH)<sub>2</sub>D<sub>3</sub>, 1,25(OH)<sub>2</sub>D<sub>3</sub>, Ca, and P<sub>i</sub>

| Dietary Ca | 1,25(OH) <sub>2</sub> D <sub>3</sub> dose | Body weight*        | Serum concentrations* of             |                      |                                       |           |                |
|------------|---|---------------------|--------------------------------------|----------------------|---------------------------------------|-----------|----------------|
|            |   |                     | 1,25(OH) <sub>2</sub> D <sub>3</sub> | 25-OH-D <sub>3</sub> | 24,25(OH) <sub>2</sub> D <sub>3</sub> | Ca        | P <sub>i</sub> |
| %          | pmol/d                                    | g                   | pg/ml                                | ng/ml                | ng/ml                                 | mg/dl     | mg/dl          |
| 0.4        | 0   | 238±4               | 151±7                                | 24±1                 | 6.8±0.9                               | 10.5±0.1  | 7.6±0.1        |
|            | 75  | 234±7               | 170±8‡                               | 12±1§                | 2.0±0.2§                              | 10.8±0.2  | 7.0±0.5        |
| 1.2        | 0   | 228±9               | 140±6                                | 24±1                 | 10.9±0.6                              | 10.9±0.1  | 7.2±0.3        |
|            | 75  | 235±7               | 199±6‡                               | 14±2§                | 3.8±0.3§                              | 12.0±0.1‡ | 6.5±0.3        |
| 2.4        | 0   | 226±7               | 121±5                                | 36±7                 | 11.4±1.1                              | 10.9±0.1  | 7.0±0.5        |
|            | 75  | 208±8 <sup>  </sup> | 182±9‡                               | 22±1§                | 3.0±0.3§                              | 11.9±0.3‡ | 5.8±0.3§       |

\* Mean±SE, six rats per group. ‡ Significantly higher than 0 dose group,  $P < 0.005$ , multivariate analysis, Tukey test. § Significantly lower than 0 dose group,  $P < 0.005$ , multivariate analysis, Tukey test. <sup>||</sup> Significantly lower than 0 dose group,  $P < 0.025$ , multivariate analysis, Tukey test.

## Discussion

Chronic  $1,25(\text{OH})_2\text{D}_3$  administration increased the serum concentration of  $1,25(\text{OH})_2\text{D}_3$  and reduced, in a dose- and time-dependent fashion, the serum concentrations of  $25\text{-OH-D}_3$  and  $24,25(\text{OH})_2\text{D}_3$ . These results confirm previous reports (8, 11) that  $1,25(\text{OH})_2\text{D}_3$  can decrease serum  $25\text{-OH-D}$  and extend these observations by defining the time course, calcium dependence, and dose-response effects of  $1,25(\text{OH})_2\text{D}_3$  on the serum concentrations of both  $25\text{-OH-D}_3$  and  $24,25(\text{OH})_2\text{D}_3$ . Furthermore, our results indicate that in the rat, the decrease in serum  $25\text{-OH-D}$  brought about by chronic  $1,25(\text{OH})_2\text{D}_3$  administration is due to an increase in the metabolic clearance of  $25\text{-OH-D}_3$  and not to a decrease in production of  $25\text{-OH-D}_3$ . Whether shorter term infusions of  $1,25(\text{OH})_2\text{D}$  will alter production of  $25\text{-OH-D}$  remains to be determined.

The increase in metabolic clearance of  $25\text{-OH-D}_3$  may in part be explained by the influence of  $1,25(\text{OH})_2\text{D}_3$  on the metabolism of  $25\text{-OH-D}_3$  in the kidney. Chronic  $1,25(\text{OH})_2\text{D}_3$  administration increased the *in vitro* conversion of  $25\text{-OH-D}_3$  to  $24,25(\text{OH})_2\text{D}_3$  in renal homogenates by approximately sevenfold, an effect consistent with previous reports demonstrating the stimulatory effect of  $1,25(\text{OH})_2\text{D}_3$  on the renal  $25\text{-hydroxyvitamin-D-24-hydroxylase}$  (24-hydroxylase) (22–27). The increase in turnover of  $25\text{-OH-D}$  to  $24,25(\text{OH})_2\text{D}$  can not be attributed to differences in tissue levels of endogenous  $25\text{-OH-D}$ . Kidney homogenate concentrations of endogenous  $25\text{-OH-D}$  in vehicle and  $1,25(\text{OH})_2\text{D}$  infused rats were 0.7 and 0.2 ng/ml, respectively, or  $1.7$  and  $0.5 \times 10^{-9}$  M, respectively, whereas the  $K_m$  for the 24-hydroxylase is  $3.0 \times 10^{-7}$  M (21). The increase in 24-hydroxylase activity would be expected to produce an increase in the turnover of  $25\text{-OH-D}_3$  to  $24,25(\text{OH})_2\text{D}_3$  *in vivo* and in turn result in a fall in circulating  $25\text{-OH-D}_3$ . If this were the only metabolic change induced by  $1,25(\text{OH})_2\text{D}_3$  administration, however, one would expect to see an increase in the serum concentration of  $24,25(\text{OH})_2\text{D}_3$ . This did not occur. In fact, the serum concentration of  $24,25(\text{OH})_2\text{D}_3$  decreased, and to a greater percentage than the serum concentration of  $25\text{-OH-D}_3$  (73% as compared with 50%, Table I, day 12). This suggests that if the fall in serum  $25\text{-OH-D}_3$  is due only to an increase in turnover to  $24,25(\text{OH})_2\text{D}_3$ , then the MCR of  $24,25(\text{OH})_2\text{D}_3$  must also be increased, a point currently under investigation in our laboratory.

It is also conceivable that  $1,25(\text{OH})_2\text{D}_3$  administration increases the metabolic turnover of  $25\text{-OH-D}_3$  through pathways other than 24-hydroxylation. For example, it is possible that  $1,25(\text{OH})_2\text{D}_3$  may increase catabolism of  $25\text{-OH-D}_3$  in the liver. This concept is supported by the report from Baran et al. indicating that  $1,25(\text{OH})_2\text{D}_3$  in the perfusate can reduce the accumulation of  $[^3\text{H}]25\text{-OH-D}_3$  in livers perfused with  $^3\text{H}$ -vitamin  $\text{D}_3$  (10). Baran et al. interpret their data to suggest that  $1,25(\text{OH})_2\text{D}_3$  decreases production of  $25\text{-OH-D}_3$ . However, their results are also consistent with a  $1,25(\text{OH})_2\text{D}_3$ -induced increase in catabolism of  $25\text{-OH-D}_3$ . Whereas our data (Table IV) provide no evidence for increased catabolism of  $25\text{-OH-D}_3$  in liver homogenates from  $1,25(\text{OH})_2\text{D}_3$  infused rats, it is possible that intact liver tissue with a functioning biliary system is required for the catabolic pathway to be operative.

Although it is clear that  $1,25(\text{OH})_2\text{D}_3$  administration increases the MCR of  $25\text{-OH-D}_3$ , it is not clear whether or not  $1,25(\text{OH})_2\text{D}_3$  acts directly to increase metabolism of  $25\text{-OH-D}_3$  or indirectly through changes in other systemic factors (e.g., cal-

cium or parathyroid hormone, PTH). Since  $1,25(\text{OH})_2\text{D}_3$  administration causes hypercalcemia, it is possible that the changes in the serum concentrations of  $25\text{-OH-D}_3$  and  $24,25(\text{OH})_2\text{D}_3$  are brought about by the hypercalcemia or the presumed decrease in circulating PTH and not the increase in serum  $1,25(\text{OH})_2\text{D}_3$  per se. To examine the influence of serum calcium on the changes in serum  $25\text{-OH-D}_3$  and  $24,25(\text{OH})_2\text{D}_3$  induced by  $1,25(\text{OH})_2\text{D}_3$ , we varied dietary calcium to modulate the changes in serum calcium brought about by  $1,25(\text{OH})_2\text{D}_3$  infusion.  $1,25\text{-Dihydroxyvitamin D}_3$  infusion increased serum calcium when dietary calcium was 1.2 and 2.4% but did not increase serum calcium when dietary calcium was 0.4%. Regardless of whether or not hypercalcemia was induced, however,  $1,25(\text{OH})_2\text{D}_3$  infusion reduced the serum concentrations of  $25\text{-OH-D}_3$  and  $24,25(\text{OH})_2\text{D}_3$ . This suggests that an increase in serum calcium is not required for  $1,25(\text{OH})_2\text{D}_3$  infusion to reduce circulating  $25\text{-OH-D}_3$  and  $24,25(\text{OH})_2\text{D}_3$ . These experiments, however, do not rule out the possibility that the presumptive fall in serum PTH, which likely occurs during  $1,25(\text{OH})_2\text{D}_3$  infusion, is in some way influencing metabolic clearance of  $25\text{-OH-D}_3$  and  $24,25(\text{OH})_2\text{D}_3$ .

In summary, we have shown that chronic  $1,25(\text{OH})_2\text{D}_3$  administration decreases the serum concentration of  $25\text{-OH-D}_3$  by increasing the metabolic clearance of  $25\text{-OH-D}_3$  and not by decreasing its production. We have also demonstrated that this phenomenon is not dependent on the induction of hypercalcemia. Furthermore, we have shown that chronic  $1,25(\text{OH})_2\text{D}_3$  administration *in vivo* increases the *in vitro* conversion of  $25\text{-OH-D}_3$  to  $24,25(\text{OH})_2\text{D}_3$  but at the same time decreases circulating  $24,25(\text{OH})_2\text{D}_3$ .

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