Extra-renal production of calcitriol in chronic renal failure

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Extra-renal production of calcitriol in chronic renal failure. Renal 1-alpha-hydroxylase activity is tightly regulated in normal humans and intact animals. No significant changes in serum $1,25(OH)_2D$ levels occur in response to vitamin D challenge. However, conflicting reports have appeared in the literature with regard to stimulation of $1,25(OH)_2D$ production after $25(OH)D$ administration in uremia. To provide further insight into this issue, $25(OH)D$ at a dose of 100 μg every other day for two weeks followed by 50 μg every other day for the next two weeks was given orally to seven uremic mongrel dogs. After two weeks of $25(OH)D$ therapy, $1,25(OH)_2D$ levels increased from 16.4 ± 0.9 to 28.0 ± 1.9 pg/ml ($P < 0.001$) in parallel with a fourfold increase in $25(OH)D$ concentrations from a basal of 50.1 ± 6.5 to 203.2 ± 18.1 ng/ml. No significant changes in serum i-PTH, ICa or P were observed. Linear regression analysis of the relationship between serum concentrations of $1,25(OH)_2D$ versus $25(OH)D$, for each dog during this period, showed highly significant correlation coefficients. To evaluate the possibility that extra-renal sites contribute to the described enhanced $1,25(OH)_2D$ net synthesis after $25(OH)D$ treatment, similar studies were performed in four anephric patients undergoing hemodialysis. Basal serum $1,25(OH)_2D$ levels were 5.5 ± 2.4 pg/ml and increased to 19.6 ± 5.0 pg/ml after $25(OH)D$ administration. A significant correlation was also found for the relationship between serum levels of $1,25(OH)_2D$ and $25(OH)D$ in anephrics ($r = 0.72$, $P < 0.001$). The same therapy in four normal volunteers showed no significant changes in serum $1,25(OH)_2D$ concentrations. Our results suggest that the effects of $25(OH)D$ administration on $1,25(OH)_2D$ levels are not exclusively a consequence of enhanced substrate availability to the renal 1-alpha-hydroxylase enzyme. Supraphysiological levels of $25(OH)D$ can augment circulating $1,25(OH)_2D$ concentrations in the absence of renal mass.

Cholecalciferol and ergocalciferol must undergo two hydroxylations, at C 25 and at C 1, to yield the biologically active metabolite $1,25(OH)_2D$ [1]. Because 1-alpha-hydroxylation is the rate limiting step in $1,25(OH)_2D$ production, it is generally assumed that regulation of this enzyme is the principal means by which changes in serum $1,25(OH)_2D$ are mediated [2–4].

This enzyme is under complex regulation by several agents, the most important being parathyroid hormone [5–11], calcium [12–16], phosphorus [17, 18] and $1,25(OH)_2D$ status of the cells or animal itself [3, 5, 6, 8]. Even though $1,25(OH)_2D$ synthesis is strictly dependent upon an adequate level of vitamin D [19–21], circulating $1,25(OH)_2D$ concentrations are not greatly affected by increased vitamin D intake. In normal adults, no significant changes in $1,25(OH)_2D$ levels have been reported either with seasonal increments of its precursor [22] or with a

15-fold increase in $25(OH)D$ in patients with vitamin D intoxication [23]. A same pattern of a tight regulation of $1,25(OH)_2D$ levels after vitamin D challenge has been also reported for chicks and rats [24]. However, an increase in serum $1,25(OH)_2D$ concentrations after $25(OH)D$ administration was found in hypoparathyroidism [25] and in normal children [26, 27]. There are also conflicting reports regarding the effects of $25(OH)D$ therapy on serum $1,25(OH)_2D$ concentrations in uremia [28–33].

Our experiments were conducted to provide further insight into the mechanisms affecting $1,25(OH)_2D$ levels in chronic renal failure.

Methods

Four normal volunteers, four anephric patients (bilateral surgical nephrectomies) undergoing hemodialysis, three normal dogs and seven dogs made uremic by 5/6 nephrectomy [34] were studied.

Baseline values were obtained without modification in dietary calcium intake in humans. In dogs, a diet providing 1.6 g Ca and 1.5 g P daily was used to prevent hypercalcemia. Baseline values were obtained three weeks after the dogs were fed the above described diet.

Dose and schedule for 25(OH)D administration

Two hundred μg per day of $25(OH)D$ were given orally for two weeks to normal and anephric humans. Normal and uremic dogs received 100 μg $25(OH)D$ every other day for two weeks. In uremic dogs, the dose was reduced to 50 μg every other day for an additional two week period. All the groups were studied for an additional two weeks after cessation of therapy. Fasting morning blood samples were drawn before $25(OH)D$ intake, once a week in humans, twice a week in normal dogs and three times a week in uremic dogs. The serum also was analyzed for total and ionized calcium, phosphorus, magnesium, potassium, sodium, creatinine, BUN, PTH, $25(OH)D$ and $1,25(OH)_2D$.

Chemical determinations

Exogenous creatinine clearances were measured in our uremic dogs at the beginning of the study using a previously described technique [34] with the dogs fasting, unanesthetized and standing in slings. Total serum calcium was measured by atomic absorption spectrometry (Model 503 Perkin Elmer Corp, Instrument Div, Norwalk, Connecticut, USA). Serum ionized calcium was measured by an ion specific flow through electrode (Model SS20, Orion Research, Inc. Cambridge, Massachusetts, USA). Serum phosphorus was measured by autoanalyzer II. (Technicon Instruments, Tarrytown, New York, USA). A

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highly sensitive aminoterminal PTH radioimmunoassay was used to measure PTH levels in dogs [35]. In humans, PTH was measured using a mid region-carboxy terminal radioimmunoassay previously described [36]. 25(OH)D was measured with a radioreceptor assay using sheep serum as the binding protein after purification of the sample using a C18 Sep Pak column [37]. 1,25(OH)$_2$D was measured using the method developed by Reinhardt [38]. In the extraction procedure, the amount of 96:4 hexane:isopropanol was doubled to assure complete elution of 25(OH)D.

Validation of 1,25(OH)$_2$D measurements

Thymus cytosolic receptor cross-reactivity for 25(OH)D is 0.1% that of 1,25(OH)$_2$D [38] but normal circulating levels of 25(OH)D are one thousand times higher than 1,25(OH)$_2$D [40, 41]. In our experiments, oral 25(OH)D administration would lead to abnormally high serum 25(OH)D levels. To check the potential interference of supraphysiological concentrations of 25(OH)D on 1,25(OH)$_2$D measurements, we added 25(OH)D to a 2 ml pool of serum to get final concentrations of 50, 150 and 300 ng/ml (recovery for 25[OH]D was 89.4 ± 3.4%). No significant changes in 1,25(OH)$_2$D measurements using Reinhardt's method were observed. 1,25(OH)$_2$D concentrations (mean ± SEM) were 41.0 ± 0.6; 42.1 ± 2.6; 40.6 ± 2.2 and 38.5 ± 4.0 for serum; serum + 50 ng/ml 25(OH)D; serum + 150 ng/ml 25(OH)D and serum + 300 ng/ml 25(OH)D, respectively. Clearly, no detectable 25(OH)D is present in the 1,25(OH)$_2$D fraction. 19nor-10 oxo-25(OH)D is a metabolite that can be produced from 25(OH)D even in the absence of cells [39, 42, 43]. It co-elutes with 1,25(OH)$_2$D using HPLC with hexane:isopropanol (89:11) as the solvent system and binds the 1,25(OH)$_2$D receptor [39, 42, 43]. A good separation of 1,25(OH)$_2$D from 19nor-10 oxo-25(OH)D can be achieved by HPLC using a Zorbax Sil column and methylene chloride:isopropanol (19:1) as the solvent system [39]. To identify elution volumes, a standard solution containing 19nor-10 oxo-25(OH)D and tritiated 1,25(OH)$_2$D in 100 µl methylene chloride:isopropanol (19:1) was chromatographed using the HPLC system described above, with the absorbance detector at a wavelength of 313 nm and a flow rate of 1 ml/min. (Fig. 1) We analyzed the contribution of 19nor-10-oxo-25(OH)D on our 1,25(OH)$_2$D measurements in nine dog samples drawn at the end of the second week receiving 25 (OH)D. 1,25(OH)$_2$D was measured using the thymus radioreceptor assay on the 1,25(OH)$_2$D fraction eluted from the Silica Sep Pak before (36.7 ± 1.9 pg/ml, N = 9) and after (33.7 ± 3.2 pg/ml, N = 9) the previously described HPLC purification to separate 19nor-10 oxo-25(OH)D. Paired t-test analysis of the differences indicated that there was no significant contribution of 19nor-10 oxo-25(OH)D to 1,25(OH)$_2$D concentrations measured with Reinhardt's method. Thus, HPLC purification of 1,25(OH)$_2$D fraction to separate 19nor-10 oxo-25(OH)D was avoided in the rest of the samples. In anephric humans, the identity of measured 1,25(OH)$_2$D was checked as follows; the usual extraction procedure was used on 2 ml serum samples. The 1,25(OH)$_2$D fraction collected from the Silica Sep Pak was dried under nitrogen, redissolved in 11% isopropanol in hexane and subjected to three subsequent HPLC purifications: a) 11% isopropanol in hexane with a Zorbax Sil column; b) 5% isopropanol in methylene chloride with a Zorbax Sil column; c) 20% water in methanol with a C18 column. In each system, the
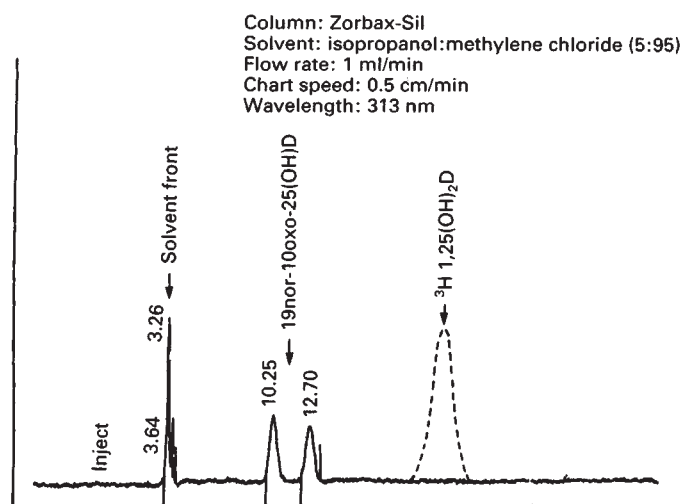

Column: Zorbax-Sil
Solvent: isopropanol:methylene chloride (5:95)
Flow rate: 1 ml/min
Chart speed: 0.5 cm/min
Wavelength: 313 nm

Fig. 1. *Elution volumes for cis and trans isomers of 19nor-10 oxo-25(OH)D (continuous line) and* ^3H *1,25(OH)$_2$D (dotted line) using HPLC.*

elution volume for 1,25(OH)$_2$D was determined with tritiated 1,25(OH)$_2$D. The 1,25(OH)$_2$D fraction was collected, dried under nitrogen and redissolved in the appropriate HPLC solvent before injection into the subsequent HPLC purification step. After the third purification, samples were dissolved in 100 µl ethanol and subjected to the radioreceptor assay described previously. The same purifications were also performed in 2 ml of our pool of normal serum containing 30 pg/ml 1,25(OH)$_2$D. The average recovery after the third HPLC purification was 56.2% ± 1.8. Although structural identification of the metabolite produced by anephric patients using mass spectrometry was not performed, (the amount of blood necessary to perform this procedure will likely jeopardize the patient's life), no significant difference (paired t-test analysis) between the 1,25(OH)$_2$D concentration before (20.7 ± 2.7 pg/ml, N = 9) and after (22.4 ± 2.7 pg/ml, N = 9) HPLC purification was observed, suggesting that our radioreceptor is measuring authentic serum 1,25(OH)$_2$D. Basal 1,25(OH)$_2$D levels in anephric patients were confirmed by Dr. DeLuca's laboratory.
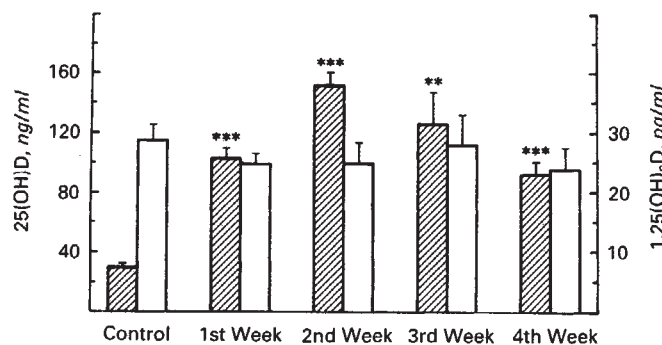
Results

Effect of 25(OH)D administration in normal dogs

Figure 2 depicts the effects of 25(OH)D administration on serum 25(OH)D and 1,25(OH)$_2$D levels in normal dogs. By the end of the second week of treatment, serum levels of 25(OH)D increased from a basal value of 33.4 ± 1.5 to 137.8 ± 8.6 ng/ml (P < 0.001) and remained three times higher than basal values even three weeks after cessation of therapy. On the other hand, 1,25(OH)$_2$D levels remained unchanged during the entire length of the study. No significant changes in the serum levels of phosphorus (basal: 3.0 ± 0.07; 1st week: 3.1 ± 0.10; 2nd week: 3.3 ± 0.14; 3rd week: 3.6 ± 0.32; 4th week: 3.0 ± 0.25 mg/dl), total calcium (basal: 10.0 ± 0.07; 1st week: 9.6 ± 0.09; 2nd week: 10.1 ± 0.06; 4th week: 9.9 ± 0.14 mg/dl) or ionized calcium (basal: 5.19 ± 0.02; 1st week: 4.93 ± 0.04; 2nd week: 5.08 ± 0.04; 3rd week: 5.16 ± 0.08; 4th week: 5.03 ± 0.06 mg/dl) or i-PTH (basal: 43.8 ± 2.7; 1st week: 57.7 ± 11.9; 2nd week:
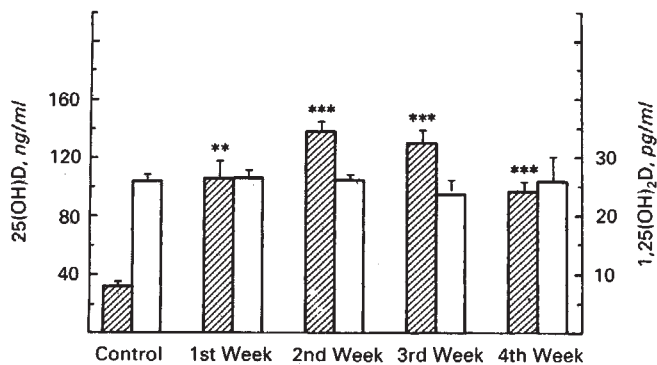
Fig. 2. *Serum 25(OH)D (▨) and 1,25(OH)₂D (□) levels before (control), and after the 1st and 2nd week of 25(OH)D administration to normal dogs. After the second week, 25(OH)D was discontinued. (Mean ± SEM). **P < 0.01; ***P < 0.001.*

Fig. 3. *Serum 25(OH)D (▨) and 1,25(OH)₂D (□) levels before (control), and after the 1st and 2nd week of 25(OH)D administration to normal humans. After the second week, 25(OH)D was discontinued. (Mean ± SEM). **P < 0.01, ***P < 0.001.*

45.7 ± 4.6; 3rd week: 65.5 ± 12.8; 4th week: 56.5 ± 7.0 pg/ml) were found. Thus, in agreement with previous reports on chicks and rats, enhanced substrate concentrations were unable to increase serum 1,25(OH)₂D concentrations in normal dogs.
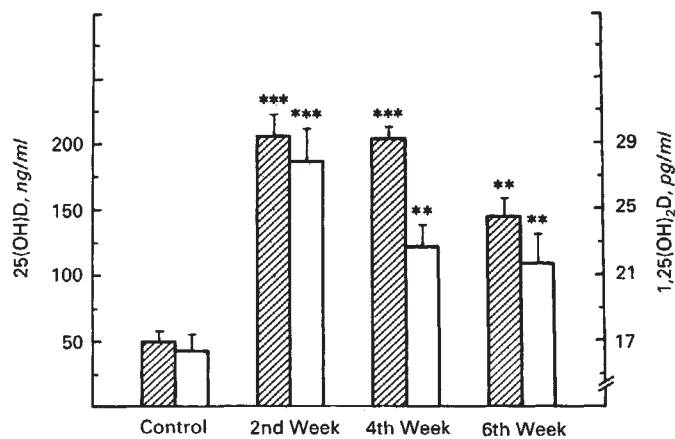
Effect of 25(OH)D administration in normal humans

Figure 3 depicts the response to 25(OH)D administration in normal volunteers.

There were significant increases in 25(OH)D concentrations from 29.0 ± 1.7 to 102.3 ± 7.8 and 152 ± 8.2 ng/ml, after one and two weeks respectively of 25(OH)D treatment.

No significant changes in 1,25(OH)₂D levels were observed.

No significant changes in i-PTH (basal: 3.1 ± 0.23; 1st week: 2.8 ± 0.25; 2nd week: 2.5 ± 0.25; 3rd week: 2.8 ± 0.25; 4th week: 2.8 ± 0.25 μEq/ml), ionized calcium (basal: 4.71 ± 0.06; 1st week: 4.94 ± 0.11; 2nd week: 4.86 ± 0.06; 3rd week: 4.76 ± 0.03; 4th week: 4.86 ± 0.06 mg/dl) or phosphorus (basal: 3.2 ± 0.19; 1st week: 3.6 ± 0.27; 2nd week: 3.5 ± 0.26; 3rd week: 3.4 ± 0.10; 4th week: 3.4 ± 0.12 mg/dl) were observed after 25(OH)D administration in normal humans. These results provide further support to the concept that serum levels of 1,25 (OH)₂D are tightly regulated in normal adults [22, 23].

Fig. 4. *Serum 25(OH)D (▨) and 1,25(OH)₂D (□) levels before (control) after the 2nd and 4th week of 25(OH)D administration to uremic dogs (N = 7). After the fourth week, 25(OH)D was discontinued. (Mean ± SEM). **P < 0.01, *** P < 0.001.*

Effects of 25(OH)D administration in uremic dogs

Figure 4 depicts the effect of 25(OH)D therapy on 25(OH)D and 1,25(OH)₂D levels in uremic dogs (GFR = 15.6 ± 1.4 ml/min).

Two weeks after treatment with 25(OH)D, serum 25(OH)D concentrations increased to four times that of basal values. In contrast to our results in normal humans and in normal dogs, serum 1,25(OH)₂D levels significantly increased from 16.4 ± 0.9 to 28.0 ± 1.9 pg/ml (P < 0.001). Both vitamin D metabolites remained significantly higher than control values even two weeks after 25(OH)D therapy was stopped.

Figures 5 and 6 show the temporal relationship between serum levels of 25(OH)D and 1,25(OH)₂D and the major modulators of 1-alpha-hydroxylase activity (PTH, ionized calcium and phosphorus). During the loading dose period (100 μg 25(OH)D every other day for two weeks), there was a parallel elevation in serum 1,25(OH)₂D levels with the increasing 25 (OH)D concentrations. In fact, linear regression analysis of the relationship between 25(OH)D and 1,25(OH)₂D for each dog revealed highly significant correlation coefficients (r_1 = 0.95, P < 0.001; r_2 = 0.80, P < 0.01; r_3 = 0.83, P < 0.01; r_4 = 0.84, P < 0.01; r_5 = 0.83, P < 0.01; r_6 = 0.84, P < 0.01; r_7 = 0.87, P < 0.01). No significant changes in i-PTH, ionized calcium or phosphorus were observed during this period suggesting that 25(OH)D, per se, increased 1,25(OH)₂D levels in uremic dogs.

During the following two weeks of receiving 50 μg of 25 (OH)D, 1,25(OH)₂D levels significantly decreased to 21.6 ± 0.8 pg/ml (still significantly higher than basal) even though 25 (OH)D concentrations remained constant. There was a slight but significant (5.31 ± 0.04 to 5.57 ± 0.6 mg/dl) increase in ionized calcium in this period which may explain the decrease in 1,25(OH)₂D concentrations without modifications in the levels of substrate.

Effects of 25(OH)D therapy on anephric patients undergoing hemodialysis

Figure 7 summarizes our results in humans. As described before, normal volunteers had a 400% increase in 25(OH)D
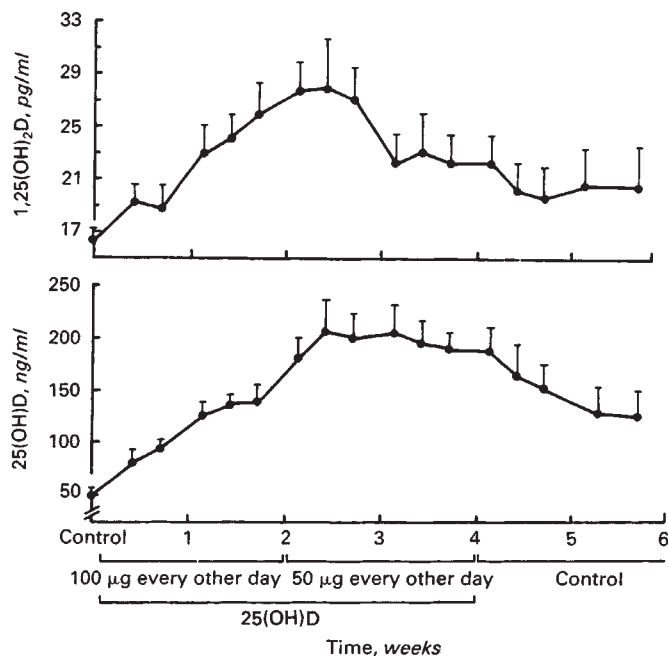
Fig. 5. *Temporal relationship between serum levels of 1,25(OH)₂D and 25(OH)D during 25(OH)D administration (0–4 weeks) and after cessation of therapy (control) in uremic dogs.* (Mean ± SEM).
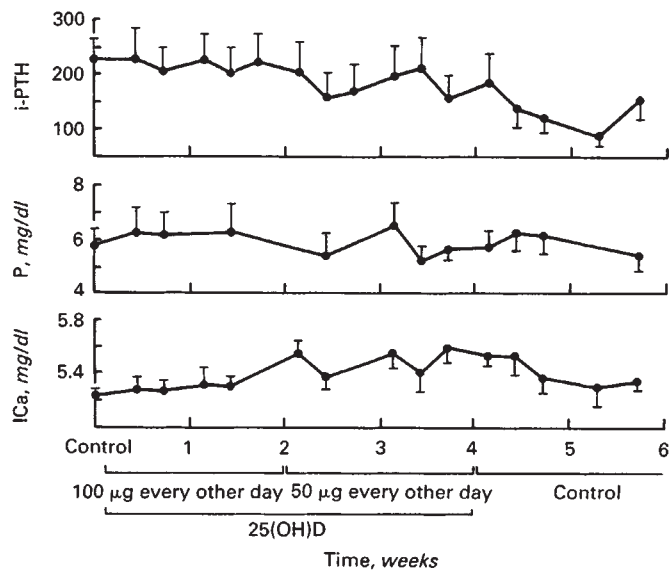

Fig. 6. *Temporal relationship between serum levels of i-PTH (pg/ml), P and ICa during 25(OH)D administration (0–4 weeks) and after cessation of therapy (control) in uremic dogs.* (Mean ± SEM).

Fig. 7. *Percent increase from basal values of serum 25(OH)D (▨) and 1,25(OH)₂D (□) during oral 25(OH)D₃ administration.* **A** Four normal adults (N = 4) and **B** four anephric patients. 25(OH)D was administered during the first two weeks. *P < 0.05, **P < 0.01, ***P < 0.001.

Fig. 8. *Linear regression analysis of the relationship between serum levels of 1,25(OH)₂D vs. 25(OH)D in anephric patients.* y = 2.76 + 0.09x; N = 18; r = 0.72; P < 0.001.

levels (basal = 29.0 ± 1.7; 1st week = 102.3 ± 7.8; 2nd week 152.2 ± 8.2; 3rd week: 125.9 ± 22.2; 4th week: 90.6 ± 6.7 ng/ml) without modifications in 1,25(OH)₂D concentrations. On the other hand, in our anephric patients, the increase in 25(OH)D levels after therapy (basal: 23.2 ± 1.8; 1st week: = 112.4 ± 16.0; 2nd week: 203.9 ± 23.4; 3rd week: 186.1 ± 23.3; 4th week: 176.4 ± 26.0 ng/ml) was accompanied by a concomitant in-
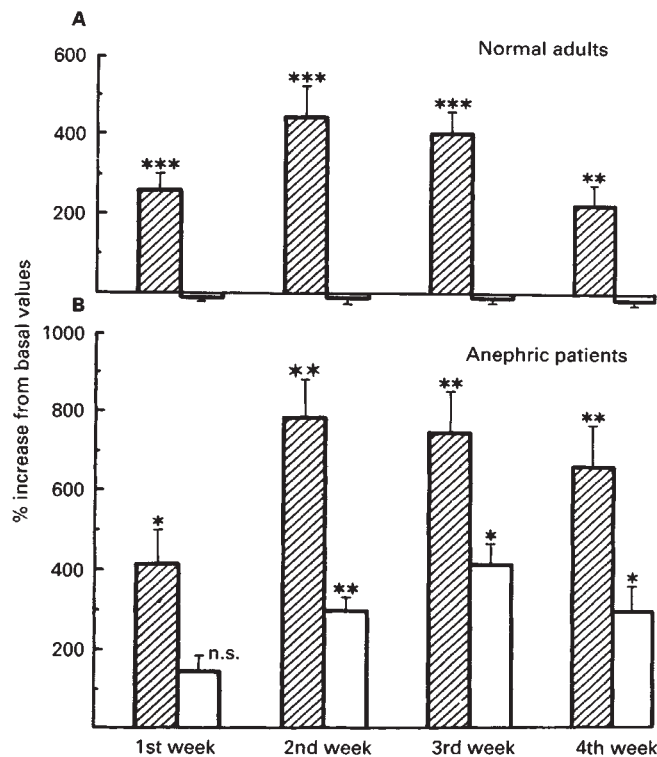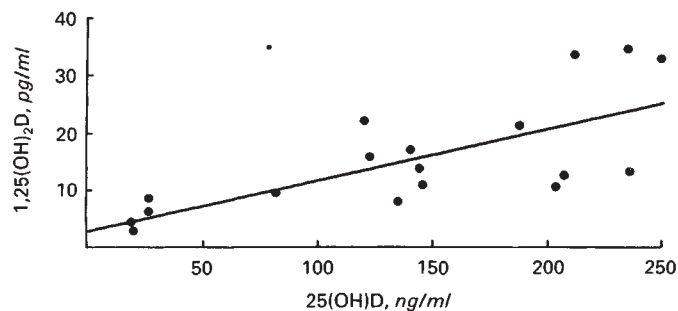
crease in 1,25(OH)₂D levels (basal: 5.5 ± 1.2; 1st week: 13.1 ± 4.6; 2nd week: 19.6 ± 4.9; 3rd week: 21.2 ± 6.3; 4th week: 18.7 ± 5.4 pg/ml). No significant changes in serum concentrations of total calcium (basal: 9.3 ± 0.3; 2nd week: 9.1 ± 0.08; 4th week: 9.5 ± 0.43 mg/dl) or ICa (basal: 4.81 ± 17; 2nd week: 4.65 ± 0.15; 4th week: 4.85 ± 0.20 mg/dl) or P (basal: 6.8 ± 0.52; 2nd week: 6.3 ± 0.33; 4th week: 7.2 ± 0.50 mg/dl) or i-PTH (basal: 318.8 ± 62.3; 2nd week: 288 ± 56.0; 4th week: 248.5 ± 65.1 mEq/ml) were observed.

Figure 8 shows a significant correlation between serum levels of 25(OH)D and 1,25(OH)₂D in anephric humans (P < 0.001).

Discussion

In agreement with previously published results for normal adults [22, 23] and other animal species (chicks and rats) [24], we observed no significant changes in serum $1,25(OH)_2D$ levels in normal adults and in normal dogs after $25(OH)D$ administration, even with a three- to fourfold increase in circulating $25(OH)D$ concentrations. Serum PTH, ICa and P levels remained unchanged, thus these modulators of 1-alpha-hydroxylase activity likely were not involved in the control of serum calcitriol levels after substrate challenge.

An increase in circulating $1,25(OH)_2D$ concentrations after vitamin D challenge has been described in cows [44], in normal children [26, 27], in hypoparathyroidism [25] and also in some cases of chronic renal failure [28, 32]. We evaluated the regulation of serum $1,25(OH)_2D$ levels in dogs with chronic renal failure.

In contrast to what was observed in normal conditions, the low basal levels of $1,25(OH)_2D$ in our uremic dogs were increased significantly after two weeks of $25(OH)D$ administration. As previously described in normal animals, no changes in serum i-PTH, ionized calcium and phosphorus levels were observed, suggesting that $25(OH)D$, per se, is responsible for the increase in $1,25(OH)_2D$ levels in uremia. This is in agreement with similar studies performed in patients with low glomerular filtration rates, which showed that serum concentrations of $1,25(OH)_2D$ were responsive to changes in the circulating levels of $25(OH)D$ [28, 32].

These effects of $25(OH)D$ were attributed to an increase in substrate availability to renal 1-alpha-hydroxylase already stimulated by high PTH levels [5–11]. We found a significant correlation for the relationship between circulating levels of $25(OH)D$ and $1,25(OH)_2D$ for each dog which seems to support this hypothesis. However, during recent years, several studies have suggested that the kidney may not be unique in metabolizing $25(OH)D$ to $1,25(OH)_2D$. Placenta [45–49], cultured bone cells [50, 51], macrophages from sarcoid tissue [52–54], LPS stimulated macrophages from normal humans [54] and also cultured normal keratinocytes [55, 56] were reported to produce a metabolite with the chromatographic properties of the hormonally active form of vitamin D. Whether such cells and tissues can contribute to circulating $1,25(OH)_2D$ levels in vivo is unclear. Hypercalcemia caused by abnormally high circulating levels of $1,25(OH)_2D$ has been demonstrated in several pathological states [57–62] as well as in patients with sarcoidosis even when severe renal failure was also present [63, 64]. The suggestion that this overproduction was extra-renal came from the report of a patient with sarcoidosis who developed renal failure and had a bilateral nephrectomy in preparation for kidney transplantation [65].

In vivo, extra-renal production of $1,25(OH)_2D$ was reported in anephric pigs after $25(OH)D$ challenge [66].

An early report from Lambert et al [67] showed detectable levels for $1,25(OH)_2D$ in anephric humans. The higher $1,25(OH)_2D$ concentrations were measured in three of these patients receiving vitamin D.

We tested the contribution of extra-renal sources in the response to $25(OH)D$ challenge by administering $25(OH)D$ to four anephric patients undergoing hemodialysis who had detectable $(5.5 \pm 1.2 \text{ pg/ml})$ basal concentrations of $1,25(OH)_2D$. They responded to $25(OH)D$ administration by a two- to threefold increase in serum $1,25(OH)_2D$ concentrations. A significant correlation between circulating levels of $25(OH)D$ and $1,25(OH)_2D$ was found in the absence of renal mass, suggesting that substrate availability to the remnant renal enzyme is not the only mechanism involved in the observed response to substrate challenge in chronic uremia.

Our results seem to support the existence of extra-renal sites with the capacity to hydroxylate the 1-alpha position. The low basal levels of circulating $1,25(OH)_2D$ found in our anephric patients reveal the poor contribution of extra-renal sources under physiological $25(OH)D$ concentrations. However, these sources were able to increase serum $1,25(OH)_2D$ levels to the lower limit of the normal range when substrate was raised to supraphysiological concentrations.

The apparent capacity of anephric humans to produce $1,25(OH)_2D$ under conditions of high precursor levels we describe in this paper, may be of value in explaining why severe hypercalcemia developed in an anephric child treated with large doses of vitamin D [68].

The requirement of supraphysiological amounts of substrate for the extra-renal enzyme may be an adequate explanation for the lack of increase in $1,25(OH)_2D$ concentrations after $25(OH)D$ therapy in two anephric patients reported by Zerwekh et al [30].

Little is known about the mechanisms operating to tightly control $1,25(OH)_2D$ levels under normal circumstances that are not present in chronic uremia. If the km reported for renal 1-alpha-hydroxylase in vitro [69, 70] are valid in vivo, serum $25(OH)D$ concentrations attained after vitamin D or $25(OH)D$ administration are within the range for an expected first order reaction, thus, it has been postulated that in normal humans increased $1,25(OH)_2D$ production by augmented substrate concentrations may be compensated by: a) an increase in serum ionized calcium, or phosphorus, or a decrease in PTH which will inhibit 1-alpha-hydroxylase. This is not a valid explanation according to our findings; b) feedback inhibition of 1-alpha-hydroxylase by physiological concentrations of $1,25(OH)_2D$; c) enhanced $1,25(OH)_2D$ catabolism to maintain calcitriol levels constant.

Feedback inhibition by physiological levels of calcitriol may be an adequate explanation for our results. Such a mechanism was described for human keratynocytes in vitro [56] and in our anephric patients, $25(OH)D$ administration didn't enhance $1,25(OH)_2D$ levels above normal range.

With regards to $1,25(OH)_2D$ catabolism, it has been described for normal humans that increased production is paralleled by enhanced catabolism to more polar metabolites mainly in the kidney to maintain mineral homeostasis [71].

The important role of the kidney in vitamin D metabolism is indicated by the lower turnover rates for $25(OH)D$ described in anephric humans [72]. This may explain the higher circulating levels of $25(OH)D$ at the second week of $25(OH)D$ administration and the slower decay after cessation of therapy in our anephric patients compared to normal humans after identical oral doses of this metabolite. Therefore, it may be postulated that in renal failure or in anephric patients decreased renal catabolism of $1,25(OH)_2D$, due to a reduction or absence of renal mass, will not compensate for the enhanced production of $1,25(OH)_2D$ after substrate challenge and will lead to the

observed increase in circulating 1,25(OH)$_2$D levels. However, recent studies performed in our laboratory [73] clearly indicate that in dogs with experimental chronic renal failure, the metabolic clearance rate of 1,25(OH)$_2$D remains unchanged. On the other hand, production rate of 1,25(OH)$_2$D$_3$ is greatly increased after 25(OH)D administration.

In summary, our results confirm the existance of tight regulation of circulating 1,25(OH)$_2$D levels in normal humans. We report that this stringent control of calcitriol concentrations is also present in normal dogs. We have demonstrated that it is possible to augment 1,25(OH)$_2$D levels in uremic dogs by oral 25(OH)D administration thus providing a good experimental model to further characterize the mechanisms involved in this diverse control of serum calcitriol.

We also demonstrate that enhanced substrate availability to renal 1-alpha-hydroxylase is not the only mechanism involved in the response to 25(OH)D therapy in chronic uremia in humans. There are extra-renal sources with the capacity to hydroxylate the 1-alpha position of cholecalciferol with poor contribution to circulating 1,25(OH)$_2$D levels under physiological 25(OH)D concentrations but with the potential to normalize serum calcitriol under conveniently elevated substrate levels.

The location and regulation of the extra-renal source/s for calcitriol in normal and uremic individuals at physiological and supraphysiological 25(OH)D concentrations need to be determined.

References

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