Oral Intake of Phosphorus Can Determine the Serum Concentration of 1,25-Dihydroxyvitamin D by Determining Its Production Rate in Humans

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Abstract

Changes in the oral intake of phosphorus could induce the reported changes in the serum concentration of 1,25-dihydroxyvitamin D (1,25-$(OH)_2D$) by inducing changes in its production rate (PR) or metabolic clearance rate (MCR), or both. To investigate these possibilities, we employed the constant infusion equilibrium technique to measure the PR and MCR of 1,25-$(OH)_2D$ in six healthy men in whom the oral intake of phosphorus was initially maintained at 1,500 mg/70 kg body weight per d for 9 d, then restricted to 500 mg/d (coupled with oral administration of aluminum hydroxide) for 10 d, and then supplemented to 3,000 mg/d for 10 d. With phosphorus restriction, the serum concentration of 1,25-$(OH)_2D$ increased by 80% from a mean of 38±3 to 68±6 pg/ml, $P < 0.001$; the PR increased from 1.8±0.2 to 3.8±0.6 μg/d, $P < 0.005$; the MCR did not change significantly. The fasting serum concentration of phosphorus decreased from 3.5±0.2 to 2.6±0.2 mg/dl, $P < 0.01$. With phosphorus supplementation, the serum concentration of 1,25-$(OH)_2D$ decreased abruptly, reaching a nadir within 2 to 4 d; after 10 d of supplementation, the mean concentration of 27±4 pg/ml was lower by 29%, $P < 0.01$, than the value measured when phosphorus intake was normal. The PR decreased to 1.3±0.2 μg/d, $P < 0.05$; the MCR did not change significantly. The fasting serum concentration of phosphorus increased significantly, but only initially. These data demonstrate that in healthy men, reductions and increases in the oral intake of phosphorus can induce rapidly occurring, large, inverse, and persisting changes in the serum concentration of 1,25-$(OH)_2D$. Changes in the PR of 1,25-$(OH)_2D$ account entirely for the phosphorus-induced changes in serum concentration of this hormone.

Introduction

1,25 dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$) is the metabolite of vitamin D currently considered to be the most biologically active with respect to enhancement of bone resorption and intestinal absorption of calcium and phosphorus (1–4). The renal synthesis of 1,25-$(OH)_2D_3$ from its endogenous precursor, 25-hydroxyvitamin D_3, is catalyzed by 25-hydroxyvitamin D_3-1α-hydroxylase (1-hydroxylase)[1] (5–8), an enzyme that can be stimulated by parathyroid hormone (PTH) (9–15) and suppressed by 1,25-$(OH)_2D$ (11, 13, 14), normal vitamin D status (15), and some function of the dietary intake of inorganic phosphorus (16), perhaps through an effect on its plasma concentration (12, 17).

Restriction of dietary phosphorus can induce an increase in the serum concentration of 1,25-$(OH)_2D$ in normal men and women (18–21) and in children with moderate renal insufficiency (22). Conversely, in patients with idiopathic hypercalciuria (23) or primary hyperparathyroidism (24), supplementation of phosphorus can induce a decrease in the serum concentration of 1,25-$(OH)_2D$ from supernormal to normal levels. It has been inferred that these phosphorus-induced changes in serum concentration of 1,25-$(OH)_2D$ reflect changes in the rate of its renal production, because in the chick and rat, restriction of dietary phosphorus can increase both the activity of renal 1-hydroxylase (16) and the synthetic rate of 1,25-$(OH)_2D_3$ (25, 26), when measured in vitro. But in a preliminary report, phosphorus restriction in the rat induced not only an increase in the plasma concentration of 1,25-$(OH)_2D$, but also a doubling of the plasma half-life of intravenously administered ^{3}H-1,25-$(OH)_2D$, suggesting that a decrease in the metabolic clearance rate (MCR) of the hormone contributed to the increase in its plasma concentration (27). Indeed, in some metabolic circumstances, a change in the plasma concentration of a steroid hormone can be accounted for, at least in part, by a change in its MCR (28–31). Thus, phosphorus-induced changes in the serum concentration of 1,25-$(OH)_2D$ might result from changes in its MCR, as well as from changes in its production rate (PR). Employing the infusion equilibrium technique, we measured these rates in healthy men after restriction and supplementation of their oral intake of phosphorus had induced clear cut and persisting increases and decreases, respectively, in the serum concentration of 1,25-$(OH)_2D$. We find that changes in the PR of 1,25-$(OH)_2D$ account entirely for the phosphorus-induced changes in serum concentration of this hormone.

Methods

We determined the effect of restriction, then supplementation, of the oral intake of phosphorus on the serum concentration, PR, and MCR of 1,25-$(OH)_2D$ in studies carried out in six healthy men (ages 26–40 yr). All studies were performed on the General Clinical Research Center under a protocol approved for use by the Committee on Human Research, University of California at San Francisco. Informed consent was obtained from each subject.

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1. *Abbreviations used in this paper:* HPLC, high pressure liquid chromatography; 1-hydroxylase, 25-hydroxyvitamin D_3-1α-hydroxylase; iPTH, immunoreactive parathyroid hormone; MCR, metabolic clearance rate; M-iPTH, midregion-iPTH; PR, production rate; PTH, parathyroid hormone.

Each subject received a constant whole food diet that provided, by calculation, 500 mg of phosphorus, 200 mg of calcium, 100 mg of magnesium, and 70 meq of sodium/70 kg body weight per d for 30 d. (All intakes are subsequently expressed per 70 kg body weight.) The intake of calcium and magnesium were maintained constant throughout by supplementing the whole food diet with orally administered calcium carbonate and magnesium sulfate (Table I). The intake of phosphorus was changed by changing the amount of phosphorus administered orally as a solution of neutral sodium and potassium phosphate (4:1 mixture of Na_2HPO_4/K_2HPO_4 and NaH_2PO_4/KH_2PO_4, 31 mg phosphorus, 0.9 meq sodium, and 0.9 meq potassium/5 ml) (Table I). During the first 9 d (5 d precontrol, 4 d control), 1,000 mg/d of supplemental phosphorus was administered in divided doses with each meal. During the next 10 d, phosphorus was restricted by replacing the sodium and potassium phosphate supplement with an equimolar amount of sodium and potassium chloride (0.9 meq sodium and 0.9 meq potassium/5 ml). Throughout this period only, aluminum hydroxide, 12 g/70 kg body weight per d, was administered in divided doses with each meal to decrease gut absorption of phosphorus. Throughout the subsequent final 10 d of study, dietary phosphorus was supplemented, 2,500 mg/d. This supplement provided 44 meq/d more sodium (and potassium) than provided by the diet and phosphate supplement during the first 19 d of the study. To keep the intakes of sodium (and potassium) constant throughout the entire study, additional sodium and potassium chloride, 44 meq/d each, was administered during the first 19 d (Table I). The basic diet provided, by calculation, 2,600 kcal/d, 9% as protein, 34% as fat, and 57% as carbohydrate.

Arterialized venous blood was drawn without stasis each day before breakfast at 8:30 a.m. for measurement of plasma concentration of ionized calcium and serum concentrations of creatinine, total calcium, phosphorus, magnesium, and 1,25-$(OH)_2D$. On the last 2 d of each dietary period, the serum concentrations of 25-hydroxyvitamin D (25-OHD) and immunoreactive parathyroid hormone (iPTH) were also measured. Spontaneously voided urine was collected daily in 24-h pools for measurement of concentrations of creatinine, calcium, phosphorus, and magnesium. On the last day of each of the three dietary periods, we employed the constant isotope infusion equilibrium technique to determine the MCR and PR of 1,25-$(OH)_2D$.

Table I. Oral Intake of Minerals during Each Period of Study

	Oral intake of phosphorus		
Mineral	Normal	Low	High
Phosphorus (*mg/d*)			
Total*	1,500	<500‡	3,000
Supplement (Na/KPO_4)	1,000	0	2,500
Calcium (*mg/d*)			
Total*	850	850	850
Supplement ($CaCO_3$)	650	650	650
Magnesium (*mg/d*)			
Total	350	350	350
Supplement ($MgSO_4$)	250	250	250
Sodium (*meq/d*)			
Total	140	140	140
Supplement (Na/KCl)	44	73	0
(Na/KPO_4)	29	0	73

Na/KPO_4 is neutral sodium and potassium phosphate; Na/KCl is sodium and potassium chloride (see Methods).

* Total intake is calculated as the sum of those minerals provided as oral supplements in the amounts indicated, and as foodstuffs containing phosphorus, calcium, magnesium, and sodium in the amounts of 500, 200, 100 mg/d, and 70 meq/d, respectively.

‡ During the period of phosphorus restriction, aluminum hydroxide, 12 g/d, was administered in divided doses with meals to decrease gut absorption of phosphorus.

Constant isotope infusion protocol. The constant infusion equilibrium method of Tait et al. (32, 33) was employed. Approximately 2.5 μCi of chromatographically purified 1,25-dihydroxy 26,27(n)-^{3}H-vitamin D_3 (^{3}H-1,25-$(OH)_2D_3$) (158 Ci/mmol, Amersham Corp., Arlington Heights, IL) in 0.5 ml absolute ethanol were solubilized in a 10-ml sterile aliquot of the patients own serum, the latter dispersed in 0.9% NaCl, and the solution infused intravenously at a constant rate of 0.193 ml/min (~4,000 dpm/min) (Harvard Apparatus Co., Inc., S. Natick, MA). To assess the possibility that isotope might stick to the infusion apparatus (glass syringe and plastic tubing) during the 20-h period of infusion, we measured counts of ^{3}H in aliquots of solution taken at the time isotope was prepared for infusion, and from the syringe and end of the plastic tubing at completion of the infusion some 21 h later. We found no evidence of significant sticking of isotope to the infusion apparatus during the period of infusion. In preliminary studies, we determined that stable serum concentrations of ^{3}H-1,25-$(OH)_2D_3$ were attained and maintained after 16 to 18 h of constant infusion of radiolabeled hormone. In subsequent studies, radiolabeled hormone was infused for 20 h; during the final 3-h equilibrium period, blood was drawn at 30-min intervals for measurement of serum concentrations of both tritiated and endogenous 1,25-$(OH)_2D$. The total amount of ^{3}H-1,25-$(OH)_2D$ administered over 20 h was ~0.005 μg, or 0.3% of the estimated daily renal production of the hormone.

Laboratory methods

Measurement of ^{3}H-1,25-$(OH)_2D$ in serum. Approximately 100 ng of chromatographically purified 1,25-$(OH)_2D_3$, kindly provided by Hoffman-La Roche, Inc., Nutley, NJ, in 20 μl of ethanol were added to each 2.0-ml serum sample to determine percentage of recovery. The serum was extracted twice with peroxide free diethyl ether (3:1, vol/vol), and the extract applied to a silica Sep-pak (Waters Inc., Milford, MA) in a solvent system of 0.1% isopropanol and 99.9% hexane. The Sep-pak was washed sequentially, first with 10 ml of isopropanol/hexane (2.5:97.5) and then with 7.5 ml of isopropanol/dichloromethane (4:96). 1,25-dihydroxyvitamin D was then eluted with 10 ml of isopropanol dichloromethane (10:90). The fraction was dried under N_2, resolubilized and applied to a radial compression separation system (Waters Inc.) using an 8-mm Si radial compression column (particle size, 5 μ) in a solvent system of isopropanol/hexane (8:92) at a flow rate of 4.5 ml/min. The content of ^{3}H-1,25-$(OH)_2D$ in the collected fraction was determined by direct scintillation counting. Recovery of 1,25-$(OH)_2D_3$ added to the serum was accomplished by comparison of the ultraviolet absorbance maximum at 254 nm of the sample to that of a series of crystalline standards of 1,25-$(OH)_2D_3$. Recovery of 1,25-$(OH)_2D_3$ added to serum was 65–70%. Intraassay coefficient of variation of ^{3}H-1,25-$(OH)_2D$ in serum was 6.8% at a tritium concentration of 150 dpm/ml.

To investigate whether tritiated metabolites of vitamin D other than ^{3}H-1,25-$(OH)_2D$ might be detected by our analytical procedures, preliminary studies were performed in which the concentration of ^{3}H-1,25-$(OH)_2D$ during equilibrium was measured using two different chromatographic procedures, straight-phase high pressure liquid chromatography (HPLC) as described, and a combination of reverse-phase followed by straight-phase HPLC. In one subject, a large volume of blood was obtained during infusion equilibrium and the serum aliquoted into 10 2.0-ml fractions. Approximately 100 ng of 1,25-$(OH)_2D_3$ were added to each 2.0-ml sample, the serum extracted twice with diethyl ether, and the extract applied to a silica Sep-pack, as described. Five of the fractions containing 1,25-$(OH)_2D$ were chromatographed using the HPLC procedure described. The other five fractions were applied first to a reverse-phase HPLC system using a μBondapak C 18 column (Waters Inc.) in a solvent system of water/methanol (25:75) at a flow rate of 2.0 ml/min, and then rechromatographed using the straight-phase radial compression separation system (Waters Inc.), as described. The content of ^{3}H-1,25-$(OH)_2D$ in the collected fractions from both procedures was determined

by direct scintillation counting. The two chromatographic procedures gave near identical values for the serum concentration of ^{3}H-1,25-$(OH)_2$D: reverse-phase followed by straight-phase, 147±2 dpm/ml, combined recovery 39±2% (recovery of reverse-phase HPLC alone not measured); straight-phase, 153±3 dpm/ml, recovery 65±3%. Accordingly, we find no evidence that the analytical procedures employed detected tritiated metabolites of vitamin D other than ^{3}H-1,25-$(OH)_2$D.

Serum concentrations of 1,25-$(OH)_2$D were measured in duplicate using a competitive protein binding assay (34) employing intestinal cytosol from normal vitamin D replete chicks. Minimum detection limits are <5 pg/assay tube; overall recovery ranged from 60 to 70%. Inter- and intraassay coefficients of variation of 1,25-$(OH)_2$D in serum were 13.4 and 12.6%, respectively, at a serum concentration of 31 pg/ml.

Serum concentrations of 25-OHD were measured as previously described (22). Serum concentrations of iPTH were measured by radioimmunoassay using two antisera: GP-IM, which has high affinity for PTH (1–84) and the midregion of the hormone, PTH (44–68), but low affinity for PTH (1–34), referred to hereafter as midregion-iPTH (M-iPTH), and CH-12M, which has high affinity for PTH (1–84), at least a 30-fold lower affinity for PTH (1–34), and no affinity for PTH (44–68) or carboxyl-terminal fragments, referred to hereafter as intact-iPTH (35). Serum and urinary concentrations of calcium and magnesium were measured by atomic absorption spectrophotometry, serum and urinary concentrations of phosphorus by a modification of the Fiske-Subbarow method (36), and urinary creatinine by auto-analyzer. Plasma ionized calcium was measured in duplicate using the Nova 8 ionized calcium analyzer (Nova Biomedical, Newton, MA).

Data analysis. The MCR of endogenous 1,25-$(OH)_2D_3$, defined as the volume of blood cleared completely and irreversibly of this hormone in unit time (33), is assumed to be equal to that of intravenously administered ^{3}H-1,25-$(OH)_2$D. At infusion equilibrium, the MCR is calculated according to the relationship (33): MCR (ml/min) = (rate of infusion of ^{3}H-1,25-$(OH)_2D_3$ [dpm/min])/(serum concentration of ^{3}H-1,25-$(OH)_2$D [dpm/ml]). The value for serum concentration of ^{3}H-1,25-$(OH)_2$D used to calculate the MCR for each subject during each period of study is the mean of four or five separate determinations of ^{3}H-1,25-$(OH)_2$D in serum obtained during equilibrium. Equilibrium was confirmed in each study by demonstrating that the slope of serum concentration of ^{3}H-1,25-$(OH)_2$D against time was not significantly different from zero. The coefficient of variation of serum ^{3}H-1,25-$(OH)_2D_3$ during equilibrium was 6.6±0.8%, n = 17.

The PR of 1,25-$(OH)_2$D is calculated according to the relationship: PR (μg/d) = MCR (ml/min) × serum concentration of endogenous 1,25-$(OH)_2$D (μg/ml) × 1,440 min/d. The value for serum concentration of endogenous 1,25-$(OH)_2$D used to calculate the PR for each subject during each period of study is the mean of three separate determinations of 1,25-$(OH)_2$D in serum obtained during the equilibrium period. All values for MCR and PR are expressed per 70 kg body weight.

Data are presented as group means±SEM. Statistical analysis was performed using repeated measurements analysis of variance; changes from control (normal dietary phosphorus) were analyzed using the paired t test. Statistical significance was defined as $P < 0.025$, after the Bonferroni correction for two comparisons (37).

Results

Serum 1,25-$(OH)_2$D. When the oral intake of phosphorus was normal, 1,500 mg/d, the serum concentration of 1,25-$(OH)_2$D in the six subjects ranged from 29 to 48 pg/ml, with the mean being 38±3 pg/ml. When phosphorus was restricted, the serum concentration of 1,25-$(OH)_2$D increased immediately and progressively, and after 6 to 8 d reached a relatively constant value of 63–75 pg/ml (Fig. 1). After 10 d of restriction, the mean value, 68±6 pg/ml, was 80% greater ($P < 0.001$) than that obtaining with a normal phosphorus intake. When phosphorus

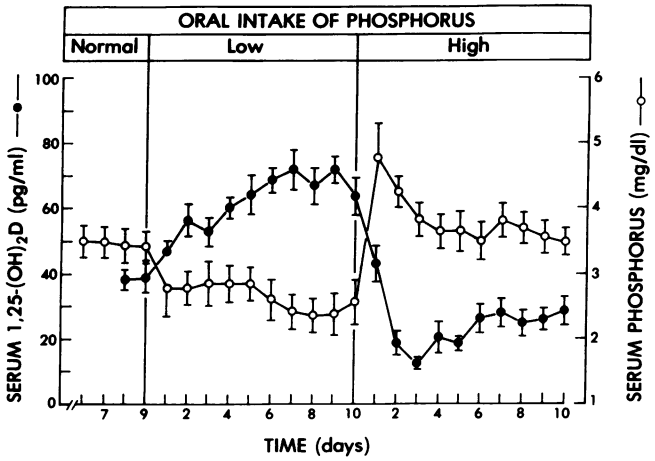

Figure 1. Effect of changes in the oral intake of phosphorus on the fasting serum concentrations of 1,25-$(OH)_2$D and phosphorus in six healthy men. The bracketed points depict mean values±SEM.

was supplemented, the serum concentration of 1,25-$(OH)_2$D decreased abruptly to a nadir of 13–18 pg/ml in 2–4 d. Over the subsequent 2–3 d, the value increased somewhat to attain a relatively constant value of 27±4 pg/ml; this value was 60% lower than that obtaining with phosphorus restriction, and 29% lower ($P < 0.01$) than that obtaining with a normal phosphorus intake. The serum concentration of 25-OHD, 13±2 ng/ml, did not change significantly when phosphorus was either restricted, 12±3 ng/ml, or supplemented, 16±4 ng/ml.

PR and MCR of 1,25-$(OH)_2$D. To determine whether the phosphorus-induced changes in serum concentration of 1,25-$(OH)_2$D were due to changes in its PR or MCR, or both, we employed the infusion equilibrium technique to estimate these rates after each subject had been maintained on each of the three intakes of phosphorus for 10 d. When the intake of phosphorus was normal, the MCR ranged from 25 to 39 ml/min per 70 kg, with a mean of 34±2 ml/min. The PR ranged from 1.3 to 2.5 μg/d per 70 kg, with a mean of 1.8±0.2 μg/d (Fig. 2).

When phosphorus was restricted, the increase in serum concentration of 1,25-$(OH)_2$D was attended by an increase in the PR in each subject; the mean value increased to 3.8±0.6 μg/d ($P < 0.005$). The metabolic clearance rate did not change significantly. When phosphorus was supplemented, the PR of 1,25-$(OH)_2$D decreased in each subject, and for the group from 3.8±0.6 to 1.3±0.2 μg/d, a decrease of 60%; the decrease from the normal phosphorus intake was 27% ($P < 0.05$). The MCR did not change significantly.

Serum phosphorus, calcium, magnesium, and iPTH. When phosphorus was restricted, the fasting serum concentration of phosphorus decreased from a mean of 3.5±0.2 to 2.6±0.2 mg/dl ($P < 0.01$) (Table II, Fig. 1). When phosphorus was supplemented, the fasting serum concentration of phosphorus increased to a maximal value after 1 d of supplementation, but then decreased so that during the final 4 d of supplementation, the mean value of 3.6±0.2 mg/dl was not significantly different from that measured with a normal phosphorus intake.

The mean serum concentrations of total and ionized calcium did not differ significantly on each of the three intakes of phosphorus; however, during the transition from the low to the high phosphorus intake, the serum concentration of ionized calcium

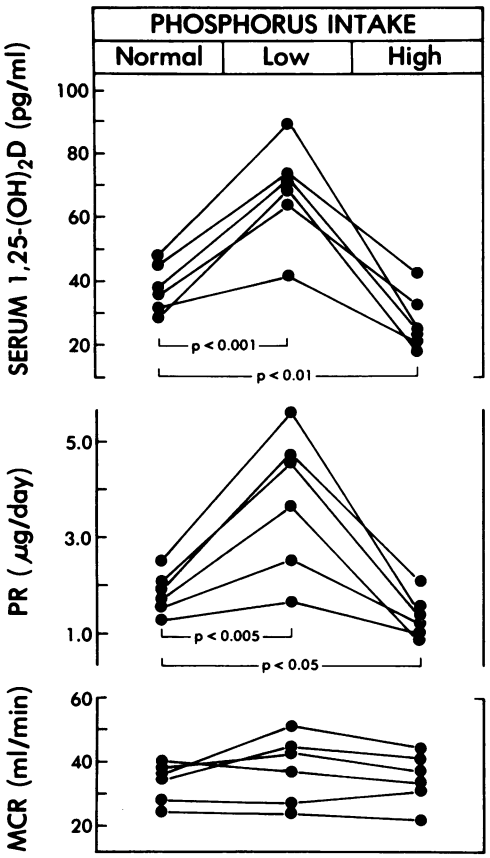

Figure 2. Effect of restriction and supplementation of phosphorus on the serum concentration, PR, and MCR of 1,25-$(OH)_2$D. The intake of phosphorus was normal for 9 d and then restricted and supplemented, consecutively, each for 10 d. Values for PR and MCR are expressed per 70 kg body weight.

decreased transiently. The serum concentration of magnesium decreased slightly but significantly when phosphorus was restricted. The serum concentrations of neither M-iPTH nor intact-iPTH differed significantly on each of the three intakes of phosphorus.

Urinary phosphorus, calcium, and magnesium. Urinary excretion of phosphorus decreased and increased rapidly in response to the decrease and increase, respectively, in phosphorus intake (Table II); renal adaptation was essentially complete within 48 h of either maneuver. Urinary excretion of calcium increased immediately and progressively after phosphorus was restricted, and after 10 d had increased by about threefold. After 10 d of phosphorus supplementation, calcium excretion was slightly but significantly lower than the control value. The changes from control (normal phosphorus intake) in urinary excretion of calcium induced by restriction and supplementation of phosphorus varied directly and significantly with the changes from control induced in serum concentration of 1,25-$(OH)_2$D, when the values in the six subjects were analyzed as a single set ($r = 0.91$, $P < 0.001$). Changes in urinary excretion of magnesium were similar in direction to, but of a lesser magnitude than, those of urinary calcium.

Discussion

The results of the present study confirm that in normal adult subjects (18–21), as in children with moderate renal insufficiency (22), restriction of the oral intake of phosphorus combined with ingestion of aluminum hydroxide induces an increase in the serum concentration of 1,25-$(OH)_2$D. When phosphorus was restricted for 10 d, the serum concentration of 1,25-$(OH)_2$D increased immediately and progressively, and within a week, reached a stable maximal value almost twice the value when phosphorus intake was normal (Fig. 1). The pattern of the increase in serum concentration of 1,25-$(OH)_2$D induced by phosphorus restriction is like that previously observed in healthy adult subjects (19–21), although the magnitude of the increase we observed (80%) is greater than that reported to occur after either 4, 10, or 18 d of phosphorus restriction (29, 46, 32%, respectively) (19–21). It seems likely that the greater increase in serum 1,25-$(OH)_2$D observed in the present study reflects a more severe restriction of dietary phosphorus (19, 21), and possibly also differences in the sex and age of study subjects (20).

The results of the present study demonstrate that in healthy men, supplementation of dietary phosphorus induces a decrease in the serum concentration of 1,25-$(OH)_2$D. When phosphorus was supplemented for 10 d (after its restriction), the serum concentration of 1,25-$(OH)_2$D decreased from a maximal value of 68 pg/ml to a nadir of ~15 pg/ml within 2 d; thereafter, the concentration increased somewhat to attain a near constant value 60% lower than that obtaining with phosphorus restriction, and ~30% lower than the value obtaining with normal phosphorus intake. These findings demonstrate that in man, persisting changes in the oral intake of phosphorus can induce rapidly occurring, large, inverse, and persisting changes in the serum concentration of 1,25-$(OH)_2$D.

We employed the isotope infusion equilibrium technique to determine whether the phosphorus-induced changes in serum concentration of 1,25-$(OH)_2$D reflected changes in its PR or MCR, or both. When phosphorus intake was normal, 1,500 mg/d, the MCR of 1,25-$(OH)_2$D was estimated to be 34±2 ml/min in the six subjects. We have previously reported that in healthy men, the endogenous serum concentration of 1,25-$(OH)_2$D varies by <20% during a 24-h period (38); in the present study the concentration of endogenous hormone was virtually constant during the 3-h infusion equilibrium period. Hence, the PR of 1,25-$(OH)_2$D can be reliably estimated from measurements of MCR and serum concentration of endogenous hormone; the mean value for production rate was 1.8±0.2 μg/d. In each of the six subjects studied, the individual values for MCR and PR were clustered tightly about their respective mean values. These mean values are in good agreement with those reported in normal adult subjects, as determined by the equilibrium infusion technique (31), and are similar to (39) or somewhat higher than (40, 41) those determined by the bolus injection technique.

When phosphorus was restricted and then supplemented, the observed increase and decrease in serum concentration of 1,25-$(OH)_2$D was associated with an increase and decrease, respectively, in the PR of the hormone, but not with a significant change in its MCR. Thus, these findings demonstrate that in healthy men, changes in the PR of 1,25-$(OH)_2$D account entirely for the phosphorus-induced changes in serum concentration of this hormone. The present findings accord with studies in the intact chick and rat in which phosphorus restriction increased the activity of 1-hydroxylase (16) and the synthetic rate of 1,25-$(OH)_2$D (25, 26), when measured in vitro in renal cortical tissue. The present studies are also consistent with previous studies in phosphorus-deprived healthy men in whom the rate of disappearance of radiolabeled 25-OH-D_3 from plasma was increased,

Table II. Changes in Blood and Urine Composition Induced by Restriction and Supplementation of Oral Intake of Phosphorus in Six Healthy Men

Phosphorus intake	Plasma ionized Ca^{++}	Serum				Urinary excretion (mg/24 h)			Ccr	Body weight
		Total Ca	Pi	Mg	M-iPTH	Intact-iPTH	Ca	Pi	Mg		
	mg/dl	*mg/dl*	*mg/dl*	*mg/dl*	*μl/eq/ml*	*pg/ml*				*ml/min*	*kg*
Normal	4.58±0.08	9.5±0.2	3.5±0.2	2.0±0.1	12±2*	108±3*	138±16	887±73	124±17	114±4	65.7±3.0
Restricted	4.58±0.1	9.4±0.2	2.6±0.2	1.8±0.04	12±2	113±9	419±60	4±2	170±21	113±4	65.5±3.0
Δ From normal	0	−0.1±0.1	−0.9±0.2	−0.2±0.03	0	+5±7	+281±53	−883±73	+46±15	−1±1	−0.2±0.1
P	NS	NS	<0.01	<0.01	NS	NS	<0.002	<0.001	<0.025	NS	NS
Supplemented	4.50±0.06	9.7±0.2	3.6±0.2	2.0±0.02	15±2	123±9	101±21	1694±115	103±21	110±4	65.4±3.1
Δ From normal	−0.08±0.06	+0.2±0.1	+0.1±0.1	0	+3±1	+15±8	−37±8	+807±46	−21±8	−4±5	−0.3±0.2
P	NS	NS	NS	NS	NS	NS	<0.005	<0.001	<0.025	NS	NS

Values are mean±SEM; *n*, six subjects. Values used in calculation of the depicted means are the average of four separate determinations made on each of the last 4 d of each dietary period, unless otherwise indicated. Ccr, creatinine clearance. * Two determinations during each dietary period.

which suggests that the rate of its conversion to 1,25-$(OH)_2$D was increased (42). The present studies accord with the proposal that in children with moderate renal insufficiency, changes in the renal production of 1,25-$(OH)_2$D underlie the reduction in its plasma concentration observed when dietary phosphorus is normal or supplemented (22, 43), and its increase to normal values observed when phosphorus is restricted (22).

The present studies do not address the question of the mechanism by which changes in the oral intake of phosphorus induce changes in the PR of 1,25-$(OH)_2$D. We observed that phosphorus restriction induced a modest but sustained decrease in the fasting serum concentration of phosphorus. Yet, Maierhofer et al. (21) reported that phosphorus restriction in healthy men induced a 30% increase in serum concentration of 1,25-$(OH)_2$D despite the absence of a sustained decrease in fasting serum concentration of phosphorus or detectably more negative phosphorus balance (21). Conversely, in the present study, during the transition from the low to high phosphorus intake when the serum level of 1,25-$(OH)_2$D decreased abruptly by ~80%, the fasting serum concentration of phosphorus nearly doubled. But fasting hyperphosphatemia was not sustained, and after 10 d of supplementation the fasting serum level of phosphorus was not higher than the control value, whereas the serum concentration and PR of 1,25-$(OH)_2$D had decreased by ~30%. Changes in phosphorus intake may induce changes in the serum concentration of phosphorus later in the day, or may induce changes in intracellular concentration or transepithelial processing of phosphorus that affect the production rate of 1,25-$(OH)_2$D even in the absence of changes in the serum concentration of phosphorus.

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References

1. Holick, M. F., M. Garabedian, and H. F. DeLuca. 1972. 1,25-Dihydroxycholecalciferol: metabolite of vitamin D_3 active on bone in anephric rats. *Science (Wash. DC).* 176:1146–1147.

2. Boyle, I. T., L. Miravet, R. W. Gray, M. F. Holick, and H. F. DeLuca. 1972. The response of intestinal calcium transport to 25-hydroxy and 1,25-dihydroxyvitamin D in nephrectomized rats. *Endocrinology.* 90:605–608.

3. Norman, A. W., and R. G. Wong. 1972. Biological activity of the vitamin D metabolite 1,25-dihydroxycholecalciferol in chickens and rats. *J. Nutr.* 102:1709–1718.

4. Chen, T. C., L. Castillo, M. Korycka-Dahl, and H. F. DeLuca. 1974. Role of vitamin D metabolites in phosphate transport of rat intestine. *J. Nutr.* 104:1056–1060.

5. Fraser, D. R., and E. Kodicek. 1970. Unique biosynthesis by kidney of biologically active vitamin D metabolite. *Nature (Lond.).* 228:764–766.

6. Gray, R. W., I. Boyle, and H. F. DeLuca. 1971. Vitamin D metabolism: the role of kidney tissue. *Science (Wash. DC).* 172:1232–1234.

7. Gray, R. W., J. L. Omdahl, J. G. Ghazarian, and H. F. DeLuca. 1972. 25-Hydroxycholecalciferol-1-hydroxylase. Subcellular location and properties. *J. Biol. Chem.* 247:7528–7532.

8. Midgett, R. J., A. M. Speilvogel, J. W. Coburn, and A. W. Norman. 1973. Studies on calciferol metabolism. VI. The renal production of the biologically active form of vitamin D, 1,25-dihydroxycholecalciferol. *J. Clin. Endocrinol. Metab.* 36:1153–1161.

9. Garabedian, M., M. F. Holick, H. F. DeLuca, and I. T. Boyle. 1972. Control of 25-hydroxycholecalciferol metabolism by parathyroid glands. *Proc. Natl. Acad. Sci. USA.* 69:1673–1676.

10. Fraser, D. R., and E. Kodicek. 1973. Regulation of 25-hydroxycholecalciferol-1-hydroxylase activity in kidney by parathyroid hormone. *Nat. New Biol.* 241:163–166.

11. Henry, H. L., R. J. Midgett, and A. W. Norman. 1974. Regulation of 25-hydroxyvitamin D_3-1-hydroxylase in vivo. *J. Biol. Chem.* 249: 7584–7592.

12. Booth, B. E., H. C. Tsai, and R. C. Morris, Jr. 1977. Parathyroidectomy reduces 25-hydroxyvitamin D_3-1α-hydroxylase activity in the hypocalcemic vitamin D-deficient chick. *J. Clin. Invest.* 60:1314–1320.

13. Henry, H. L. 1979. Regulation of the hydroxylation of 25-hydroxyvitamin D_3 in vivo and in primary cultures of chick kidney cells. *J. Biol. Chem.* 254:2722–2729.

14. Tanaka, Y., and H. F. DeLuca. 1984. Rat renal 25-hydroxyvitamin D_3 1- and 24-hydroxylases: their in vivo regulation. *Am. J. Physiol.* 246:E168–E173.

15. Booth, B. E., H. C. Tsai, and R. C. Morris, Jr. 1985. Vitamin D status regulates 25-hydroxyvitamin D_3-1α-hydroxylase and its responsiveness to parathyroid hormone in the chick. *J. Clin. Invest.* 75:155–161.

16. Baxter, L. A., and H. F. DeLuca. 1976. Stimulation of 25-hydroxyvitamin D_3-1α-hydroxylase by phosphate depletion. *J. Biol. Chem.* 251:3158–3161.

17. Tanaka, Y., and H. F. DeLuca. 1973. The control of 25-hydroxyvitamin D metabolism by inorganic phosphorus. *Arch. Biochem. Biophys.* 154:566–574.

18. Gray, R. W., D. R. Wilz, A. E. Caldas, and J. Lemann, Jr. 1977. The importance of phosphate in regulating plasma 1,25-$(OH)_2$-vitamin D levels in humans: studies in healthy subjects, in calcium-stone formers and in patients with primary hyperparathyroidism. *J. Clin. Endocrinol. Metab.* 45:299–306.

19. Insogna, K. L., A. E. Broadus, and J. M. Gertner. 1983. Impaired phosphorus conservation and 1,25-dihydroxyvitamin D generation during phosphorus deprivation in familial hypophosphatemic rickets. *J. Clin. Invest.* 71:1562–1569.

20. Lufkin, E. G., R. Kumar, and H. Heath III. 1983. Hyperphosphatemic tumoral calcinosis: effects of phosphate depletion on vitamin D metabolism, and of acute hypocalcemia on parathyroid hormone secretion and action. *J. Clin. Endocrinol. Metab.* 56:1319–1322.

21. Maierhofer, W. J., R. W. Gray, and J. Lemann, Jr. 1984. Phosphate deprivation increases serum 1,25-$(OH)_2$-vitamin D concentrations in healthy men. *Kidney Int.* 25:571–575.

22. Portale, A. A., B. E. Booth, B. P. Halloran, and R. C. Morris, Jr. 1984. Effect of dietary phosphorus on circulating concentrations of 1,25-dihydroxyvitamin D and immunoreactive parathyroid hormone in children with moderate renal insufficiency. *J. Clin. Invest.* 6:1580–1589.

23. Van den Berg, C. J., R. Kumar, D. M. Wilson, H. Heath III, and L. H. Smith. 1980. Orthophosphate therapy decreases urinary calcium excretion and serum 1,25-dihydroxyvitamin D concentrations in idiopathic hypercalciuria. *J. Clin. Endocrinol. Metab.* 51:998–1001.

24. Broadus, A. E., J. S. Magee, L. E. Mallette, R. L. Horst, R. Lang, P. S. Jensen, J. M. Gertner, and R. Baron. 1983. A detailed evaluation of oral phosphate therapy in selected patients with primary hyperparathyroidism. *J. Clin. Endocrinol. Metab.* 56:953–961.

25. Gray, R. W., and J. L. Napoli. 1983. Dietary phosphate deprivation increases 1,25-dihydroxyvitamin D_3 synthesis in rat kidney in vitro. *J. Biol. Chem.* 258:1152–1155.

26. Lobaugh, B., and M. K. Drezner. 1983. Abnormal regulation of renal 25-hydroxyvitamin D-1-hydroxylase activity in the X-linked hypophosphatemic mouse. *J. Clin. Invest.* 71:400–403.

27. Pollard, S. K., and A. D. Kenny. 1982. Effect of low phosphorus diet on the elimination rate of 1,25-dihydroxyvitamin D_3 in male rats. *Calcif. Tissue Int.* 34:S58. (Abstr.)

28. Dluhy, R. G., L. Axelrod, R. H. Underwood, and G. H. Williams. 1972. Studies of the control of plasma aldosterone concentration in normal man. *J. Clin. Invest.* 51:1950–1957.

29. Hulter, H. N., A. Sebastian, J. F. Sigala, J. H. Licht, R. D. Glynn, M. Schambelan, and E. G. Biglieri. 1980. Pathogenesis of renal hyperchloremic acidosis resulting from dietary potassium restriction in the dog: role of aldosterone. *Am. J. Physiol.* 238:F79–F91.

30. Gordon, G. G., A. L. Southren, S. Tochimoto, J. J. Rand, and J. Olivo. 1969. Effect of hyperthyroidism and hypothyroidism on the metabolism of testosterone and androstenedione in man. *J. Clin. Endocrinol. Metab.* 29:164–170.

31. Insogna, K., A. Broadus, B. Dreyer, and J. Gertner. 1983. Infusion equilibrium measurement of 1,25-$(OH)_2$D vitamin D kinetics in normal subjects and disorders of mineral metabolism. *Clin. Res.* 31:388. (Abstr.)

32. Tait, J. F., B. Little, S. A. S. Tait, and C. Flood. 1962. The metabolic clearance rate of aldosterone in pregnant and nonpregnant subjects estimated by both single-injection and constant-infusion methods. *J. Clin. Invest.* 12:2093–2100.

33. Tait, J. F. 1963. Review: the use of isotopic steroids for the measurement of production rates in vivo. *J. Clin. Endocrinol. Metab.* 23: 1285–1297.

34. Shepard, R. M., R. L. Horst, A. J. Hamstra, and H. F. DeLuca. 1979. Determination of vitamin D and its metabolites in plasma from normal and anephric man. *Biochem. J.* 182:55–69.

35. Gallagher, J. C., B. L. Riggs, C. M. Jerpbak, and C. D. Arnaud. 1980. The effect of age on serum immunoreactive parathyroid hormone in normal and osteoporotic women. *J. Lab. Clin. Med.* 95:373–385.

36. Fiske, C. H., and Y. Subbarow. 1925. The colorimetric determination of phosphorus. *J. Biol. Chem.* 66:375–400.

37. Wallenstein, S., C. L. Zucker, and J. L. Fleiss. 1980. Some statistical methods useful in circulation research. *Circ. Res.* 47:1–9.

38. Halloran, B. P., A. A. Portale, M. Castro, R. C. Morris, Jr., and R. S. Goldsmith. 1985. Serum concentration of 1,25-dihydroxyvitamin D in the human: diurnal variation. *J. Clin. Endocrinol. Metab.* 60:1104–1110.

39. Seeman, E., R. Kumar, G. G. Hunder, M. Scott, H. Heath III, and B. L. Riggs. 1980. Production, degradation, and circulating levels of 1,25-dihydroxyvitamin D in health and in chronic glucocorticoid excess. *J. Clin. Invest.* 66:664–669.

40. Gray, R. W., A. E. Caldas, D. R. Wilz, J. Lemann, Jr., G. A. Smith, and H. F. DeLuca. 1978. Metabolism and excretion of ^{3}H-1,25-$(OH)_2$-Vitamin D_3 in healthy adults. *J. Clin. Endocrinol. Metab.* 46: 756–765.

41. Maierhofer, W. J., R. W. Gray, N. D. Adams, G. A. Smith, and J. Lemann, Jr. 1981. Synthesis and metabolic clearance of 1,25-dihydroxyvitamin D as determinants of serum concentration: a comparison of two methods. *J. Clin. Endocrinol. Metab.* 53:472–475.

42. Dominguez, J. H., R. W. Gray, and J. Lemann, Jr. 1976. Dietary phosphate deprivation in women and men: effects on mineral and acid balances, parathyroid hormone and the metabolism of 25-OH-vitamin D. *J. Clin. Endocrinol. Metab.* 43:1056–1068.

43. Portale, A. A., B. E. Booth, H. C. Tsai, and R. C. Morris, Jr. 1982. Reduced plasma concentration of 1,25-dihydroxyvitamin D in children with moderate renal insufficiency. *Kidney Int.* 21:627–632.