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Dietary intake of phosphorus modulates the circadian rhythm in serum concentration of phosphorus. Implications for the renal production of 1,25-dihydroxyvitamin D.

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Research Article

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Dietary Intake of Phosphorus Modulates the Circadian Rhythm in Serum Concentration of Phosphorus

Implications for the Renal Production of 1,25-Dihydroxyvitamin D

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Abstract

We recently reported that in healthy men, changes in the production rate (PR) of 1,25-dihydroxyvitamin D [1,25-$(OH)_2$D] accounted for the 80% increase and the 30% decrease in its serum concentration that was induced by restriction and supplementation, respectively, of dietary phosphorus. These changes in PR and serum concentration of 1,25-$(OH)_2$D could be mediated by changes in serum concentrations of phosphorus that occur after the morning fasting period. To examine this hypothesis, we measured serum concentrations of phosphorus in blood drawn at hourly intervals for 24 h in six healthy men in whom dietary phosphorus was initially maintained at 1,500 mg/70 kg body weight per day for 9 d, then restricted to 500 mg/d (coupled with orally administered aluminum hydroxide) for 10 d, and then supplemented to 3,000 mg/d for 10 d. When dietary phosphorus was normal, the serum concentration of phosphorus exhibited the normal circadian rhythm: a rapid decrease in early morning to a nadir at 1100, followed by an increase to plateau at 1600 h and a further increase to an acrophase (peak) at 0030 h. The variation in serum levels of phosphorus can be described as the sum of sinusoidal functions with periodicities of 24 and 12 h. Phosphorus restriction for 10 d induced a 40% reduction in the 24-h mean serum level of phosphorus, abolished the early afternoon rise in its serum level (i.e., the 12-h periodic component of the time series), and delayed the acrophase by 3 h to 0330 h. Phosphorus supplementation for 10 d induced a 14% increase in the 24-h mean serum level of phosphorus but no significant change in its morning fasting level, exaggerated the early afternoon rise in serum phosphorus, and advanced the acrophase by 9 h to 1530 h. The changes in the PR of 1,25-$(OH)_2$D induced by restriction and supplementation of dietary phosphorus varied inversely and significantly with those induced in the 24-h mean serum level of phosphorus ($R = -0.88$, $P < 0.001$). These data demonstrate that in healthy men, dietary phosphorus is an important determinant of the serum concentration of phosphorus throughout most of the day. The data suggest that diet-induced changes in serum levels of phosphorus mediate the changes in PR and serum concentration of 1,25-$(OH)_2$D.

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Introduction

The renal synthesis of 1,25-dihydroxyvitamin D [1,25-$(OH)_2$D], the most biologically active metabolite of vitamin D known, is catalyzed by 25-hydroxyvitamin D-1α-hydroxylase (1-hydroxylase) (1–7), an enzyme that can be stimulated by parathyroid hormone (PTH)[1] (8–14), and suppressed by 1,25-$(OH)_2$D (10, 12, 13, 15), normal vitamin D status (14), blood ionized calcium (10, 16–18), and some function of the dietary intake of inorganic phosphorus (19, 20). When dietary phosphorus is restricted in normal adult subjects (21–25) and in patients with moderate renal insufficiency (26, 27), the serum concentration of 1,25-$(OH)_2$D increases. Conversely, when dietary phosphorus is supplemented in normal men (25) and patients with idiopathic hypercalciuria (28) or primary hyperparathyroidism (29), the serum concentration of 1,25-$(OH)_2$D decreases. We have shown in healthy men that these phosphorus-induced changes in serum concentration of 1,25-$(OH)_2$D can be accounted for entirely by changes in the production rate (PR) of the hormone (25). The mechanism by which changes in dietary phosphorus induce changes in the PR of 1,25-$(OH)_2$D has, however, not been defined. In the chick and rat, the concentration of phosphorus in serum (11, 19, 30, 31) or bathing medium (32) can be an important determinant of the activity of 1-hydroxylase and the production rate of 1,25-$(OH)_2$D. Yet, in humans, both restriction (24) and supplementation (25, 28) of dietary phosphorus can induce sustained changes in serum concentration of 1,25-$(OH)_2$D without sustained changes in the morning fasting serum concentration of phosphorus. These observations raise the question of whether changes in serum concentration of phosphorus do in fact mediate the changes in serum concentration of 1,25-$(OH)_2$D induced by manipulation of dietary phosphorus.

In normal subjects ingesting a normal diet, the serum concentration of phosphorus exhibits a circadian rhythm that is characterized by a nadir in the morning, a rise in early afternoon, and a peak at night (33–37). Indeed, Stanbury reported such a circadian rhythm in serum phosphorus in normal adult subjects ingesting only a small, constant amount of fluid hourly for 24 h (33). But Jubiz et al. subsequently reported that the nocturnal peak in serum phosphorus level was abolished in normal subjects receiving neither food nor fluid for 24 h, and suggested that the circadian changes in serum phosphorus were due in large part to food ingestion (35). In fact, it is not known whether the circadian rhythm in serum phosphorus can be affected by changes only in phosphorus intake in sub-

1. *Abbreviations used in this paper:* iPTH, immunoreactive parathyroid hormone; MCR, metabolic clearance rate; PR, production rate; PTH, parathyroid hormone.

jects ingesting an otherwise normal and constant diet. Thus, changes in the PR and serum concentration of 1,25-$(OH)_2D$ induced by restriction and supplementation of dietary phosphorus might be mediated by changes in serum levels of phosphorus that occur after the morning fasting period. The results of the present study demonstrate that dietary phosphorus is an important determinant of the circadian rhythm in serum concentration of phosphorus, and suggest that diet-induced changes in serum levels of phosphorus mediate the changes in PR and serum concentration of 1,25-$(OH)_2D$.

Methods

We studied six healthy men (ages 26–40 yr) to determine the effect of restricting and then supplementing the oral intake of phosphorus on the circadian rhythms of serum phosphorus and calcium, and on the serum concentration, PR, and metabolic clearance rate (MCR) of 1,25-$(OH)_2D$. All studies were performed at 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. A portion of the data from this study has previously been published (25).

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 per 70 kg body weight/d for 30 d. (Dietary intakes are subsequently expressed per 70 kg body weight.) The intakes of calcium and magnesium were maintained constant at 850 and 350 mg/d, respectively, by supplementing the diet with orally administered calcium carbonate and magnesium sulfate. The intake of phosphorus was changed by changing the amount of supplemental phosphorus administered as 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 per 5 ml). For the first 9 d, 1,000 mg/d of supplemental phosphorus was administered orally in divided doses with meals. For 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 per 5 ml), and by administering aluminum hydroxide, 12 g/70 kg body weight/d, in divided doses with each meal. Throughout the subsequent final 10 d, dietary phosphorus was supplemented with 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. The basic diet provided, by calculation, 2,600 kcal/d, 9% as protein, 34% as fat, and 57% as carbohydrate. Meals were offered each day at 0900, 1230, and 1715 h.

On the 9th d of normal phosphorus intake, on the 1st and 10th d of phosphorus restriction, and on the 10th d of its supplementation, blood was drawn from an indwelling venous catheter at 1-h intervals for 24 h beginning at 0800 for measurement of serum concentrations of phosphorus and total calcium, and at 1–2-h intervals from 0800 to 1600 for measurement of blood concentrations of ionized calcium. (Due to technical reasons, in one subject studied during phosphorus supplementation, blood was drawn for only 8 h; i.e., from 0800 to 1600.) During the same 24-h period, we determined the MCR and PR of 1,25-$(OH)_2D$, using the equilibrium infusion technique (38, 39), as we described previously in detail (25). In brief, ~ 3 μCi of chromatographically purified [^{3}H]1,25-$(OH)_2D_3$ (158 Ci/mmol, Amersham Corp., Arlington Heights, IL) was infused intravenously at a constant rate (~ 4,000 dpm/min) for 20 h; during the final 3-h equilibrium period (0900 to 1200), blood was drawn at 30-min intervals for measurement of serum concentrations of both tritiated and endogenous 1,25-$(OH)_2D$. Spontaneously voided urine was collected in 2-h pools for 26 h beginning at 0700 for measurement of concentrations of phosphorus, calcium, and creatinine. On the last 2 d of each dietary period, the morning fasting serum concentrations of 25-hydroxyvitamin D (25-OHD) and immunoreactive parathyroid hormone (iPTH) were measured, and the 24-h urinary excretion of cyclic adenosine monophosphate (cAMP) determined.

Laboratory methods. The serum concentration of [^{3}H]1,25-$(OH)_2D$ was measured as previously described (25). 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. Serum concentrations of endogenous 1,25-$(OH)_2D$ were measured in duplicate using a competitive protein binding assay (40) employing intestinal cytosol from normal vitamin D–replete chicks. Minimum detection limits are < 5 pg per assay tube; overall recovery ranged from 60 to 70%. Inter- and intraassay coefficients of variation of 1,25-$(OH)_2D$ 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 (26). Serum concentrations of iPTH were measured by radioimmunoassay using two antisera: GP-1M, which has high affinity for PTH (1–84) and the mid-region of the hormone, PTH (44–68), but low affinity for PTH (1–34), referred to hereafter as mid-region 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 (41). Serum and urinary concentrations of calcium were measured by atomic absorption spectrophotometry, serum and urinary concentrations of phosphorus by a modification of the Fiske-Subbarow method (42), urinary concentration of creatinine by autoanalyzer, and urinary concentration of cAMP by radioimmunoassay (Immuno Nuclear Corp., Stillwater, MN). Whole blood concentrations of ionized calcium were measured in triplicate using an ionized calcium/pH analyzer (Nova 8; Nova Biomedical, Newton, MA). The within-day (n = 17) and between-day (n = 40) coefficients of variation of ionized calcium determined using aqueous controls were < 2%.

Data analysis. The MCR of endogenous 1,25-$(OH)_2D_3$ is assumed to be equal to that of intravenously administered [^{3}H]1,25-$(OH)_2D_3$. At infusion equilibrium, the MCR is calculated according to the relationship (39):

MCR (milliliters per minute) = (rate of infusion of [^{3}H]1,25-$(OH)_2D_3$ [disintegrations per minute per minute])/(serum concentration of [^{3}H]1,25-$(OH)_2D$ [disintegrations per minute per milliliter]).

The value for serum concentration of [^{3}H]1,25-$(OH)_2D$ 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-$(OH)_2D$ in serum obtained during equilibrium. Equilibrium was confirmed in each study by demonstrating that the slope of serum concentration of [^{3}H]1,25-$(OH)_2D$ against time was not significantly different from zero. The coefficient of variation of serum [^{3}H]1,25-$(OH)_2D$ for the six subjects during equilibrium was 6.6±0.8% (n = 17).

The PR of 1,25-$(OH)_2D$ is calculated according to the relationship:

PR (micrograms per day) = MCR (milliliters per minute) × serum concentration of endogenous 1,25-$(OH)_2D$ (micrograms per milliliter) × 1,440 min/d.

The value for serum concentration of endogenous 1,25-$(OH)_2D$ used to calculate the PR for each subject during each period of study is the mean of three separate determinations of 1,25-$(OH)_2D$ in serum obtained during the equilibrium period. In normal adult subjects, the serum concentration of 1,25-$(OH)_2D$ is maintained within narrow limits throughout the day (37, 43), varying by < 20% of its 24-h mean level (37). This strongly suggests that the PR of the hormone is nearly constant throughout the day. Accordingly, the PR of 1,25-$(OH)_2D$, estimated between 0900 and 1200 using the equilibrium infusion technique, appears to provide a reliable estimate of the PR of the hormone

throughout the day under steady state conditions. All values for MCR and PR are expressed per 70 kg body weight.

Time series analysis. The serum concentrations of phosphorus and calcium, measured throughout each 24-h period on each of the three intakes of phosphorus, were analyzed in a manner similar to that recommended by Van Cauter (44). The 24-h mean serum concentration of each mineral was calculated for each subject for each 24-h period studied. The variations in concentration over time (time series) for each individual were tested against the hypothesis of their random occurrence using the autocorrelation function (45). Using a computer program provided as part of the SAS System (SAS Institute Inc., Cary, NC), we then subjected each time series to spectral analysis (45, 46), a technique using the finite Fourier transform to search for periodic trends in data. The time series is described as a sum of sinusoidal functions of different amplitudes and periodicities (periodogram calculation). If, for each time series, the hypothesis of random variation was rejected, based upon either the autocorrelation function or Bartlett's Kolmogorov-Smirnov test (the latter based upon the normalized cumulative periodogram [46]), those periodicities that contribute significantly to the observed variation were selected using a test procedure described by Fuller et al. with a minimum probability of 90% (46). The significant periodic components are used to construct a theoretical curve that describes the data. The circadian acrophase and nadir are, respectively, the times of occurrence of maxima and minima of the theoretical curve; its circadian amplitude is calculated as one-half the difference between its maximum and minimum values, and is expressed in absolute concentration units (absolute amplitude) or as a percentage of the 24-h mean level (relative amplitude).

Data are presented as group means±SEM. Statistical analysis was performed using repeated-measures analysis of variance; changes from the normal phosphorus period were analyzed using the paired t test using the Bonferroni correction for two comparisons (47). Correlation coefficients were calculated by the method of least squares.

Results

Serum phosphorus. When dietary phosphorus was normal, the serum concentration of phosphorus exhibited a circadian rhythm like that previously described in healthy men eating three meals per day at similar times (36, 37): a rapid decrease in early morning reaching a nadir of 3.3±0.3 mg/dl at 1100, followed by an increase to a plateau at 1600, and a further increase to a peak of 4.6±0.2 mg/dl at 0100 to 0300 (Fig. 1). Spectral analysis of each individual time series demonstrated that a significant nocturnal peak was present at ~ 0100 in each subject, and a significant afternoon peak at ~ 1400 in five of the six subjects. Thus, the time series from each subject demonstrated the presence of significant periodicities of 24 h, 12 h, or both. These significant periodic components were used to construct a theoretical curve of serum phosphorus concentration as a function of time for each subject. The mean circadian amplitude, calculated from the theoretical curves, was 0.6±0.1 mg/dl (absolute), or 15.2±2.6% (relative). The circadian acrophase (peak) occurred between 0100 and 0200 in five of the six subjects, and at 2100 in the other. The mean acrophase for the group occurred at 0030±40 min.

Serum levels of phosphorus were measured throughout the first 24 h of its dietary restriction. At 1000, 1 h after initiating phosphorus restriction, the serum concentration of phosphorus decreased significantly (delta 0.5±0.2 mg/dl, $P < 0.02$) and remained decreased throughout the day (Fig. 1). By the morning after initiation of restriction, the concentrations of phosphorus had returned toward their previous levels, although the value at 0800 was slightly but significantly lower ($P < 0.05$)

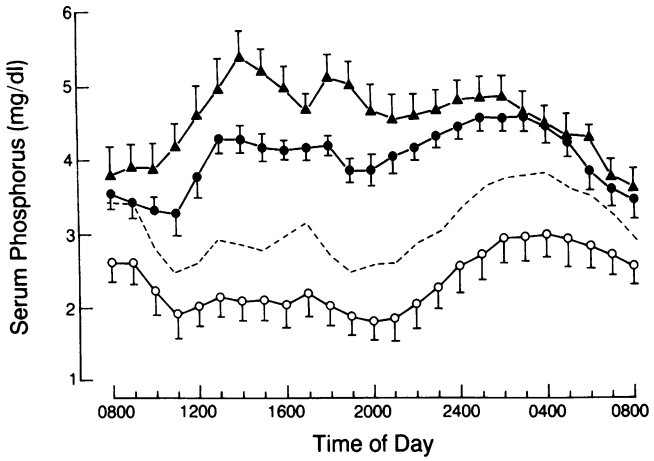

Figure 1. Effect of dietary phosphorus on the circadian rhythm in serum phosphorus concentration in healthy men. Blood was drawn from an indwelling venous needle at hourly intervals for 24 h beginning at 0800, after each subject had received the normal intake (●) (1,500 mg/d) of phosphorus for 9 d, and after dietary phosphorus was restricted for 1 d (---) and 10 d (○) (< 500 mg/d), and then supplemented (▲) (3,000 mg/d) for 10 d. The variations in serum phosphorus concentration over time (time series) for each individual were subjected to spectral analysis, in which the time series is described as a sum of sinusoidal functions of different amplitudes and periodicities. In each subject, significant periodicities of 24 h, 12 h, or both were demonstrated. Depicted are mean values±SEM.

than that measured when phosphorus intake was normal. Phosphorus restriction for 10 d induced a 23% decrease in the morning fasting serum level of phosphorus, and abolished the rise in serum phosphorus that normally occurs in the early afternoon, such that the 24-h mean level decreased by 40% (Fig. 1, Table I). The magnitude of the fall in serum phosphorus level at 1600 was twice that at 0800. Even with phosphorus restriction, the nocturnal peak in phosphorus concentration was present in each subject, the 24-h periodicity being confirmed by spectral analysis; however, the early afternoon rise, i.e., the 12-h periodic component, disappeared. The circadian amplitude, either absolute, 0.5±0.1 mg/dl, or relative, 23.4±2.3%, was not significantly different from that when dietary phosphorus was normal. The acrophase was shifted later by 3 h to 0330 ($P < 0.05$).

Supplementation of dietary phosphorus for 10 d induced no significant change in morning fasting serum levels of phosphorus, but exaggerated the rise in serum phosphorus in the afternoon and evening, so that the 24-h mean serum concentration increased significantly (Fig. 1, Table I). In each subject studied, the major peak in phosphorus concentration was observed in late afternoon, the mean circadian acrophase occurring at 1530, 9 h earlier ($P < 0.001$) than when dietary phosphorus was normal. Three of the subjects also demonstrated a significant, but lower, nocturnal peak in phosphorus concentration. There was no significant change in the circadian amplitude with phosphorus supplementation.

Total and ionized calcium. When dietary phosphorus was normal, the serum concentration of total calcium exhibited periodic variation like that previously described in healthy men (36, 37), with a peak of 9.7±0.1 mg/dl occurring at ~ 1300 and a nadir of 9.1±0.2 mg/dl at 0300. Spectral analysis demonstrated the presence of a significant periodicity of

Table I. Changes in Blood and Urine Composition Induced by Restriction and Supplementation of Dietary Phosphorus in Six Healthy Men

	Serum phosphorus		Serum total calcium		Blood ionized calcium		
Phosphorus intake	Fasting	24-h mean	Fasting	24-h mean	Fasting	"Day" mean	Urinary excretion of cAMP
	mg/dl		*mg/dl*		*mg/dl*		*nmol/100 ml GF*
Normal	3.6±0.3	4.0±0.2	9.6±0.4	9.4±0.1	4.55±0.07	4.48±0.05	2.45±0.24
Restricted	2.6±0.7	2.3±0.3	9.6±0.5	9.4±0.1	4.60±0.10	4.57±0.08	1.84±0.20
ΔFrom normal	−1.0±0.5	−1.7±0.4	0	0	0.05±0.10	0.09±0.08	−0.62±0.10
P	<0.01	<0.005	NS	NS	NS	NS	<0.001
Supplemented	3.7±0.7	4.6±0.3*	9.7±0.5	9.4±0.1*	4.45±0.09	4.34±0.08	2.43±0.16
ΔFrom normal	0.1±0.2	0.6±0.1	0.1±0.1	0	−0.10±0.06	0.14±0.05	−0.01±0.09
P	NS	<0.001	NS	NS	NS	<0.05	NS

Values are mean±SEM. * *N*, five subjects. GF, Glomerular filtrate.

either 24 or 12 h in the time series of three of the six subjects. Restriction and supplementation of dietary phosphorus induced no significant change in either the fasting or the 24-h mean serum concentration of total calcium (Table I).

Markowitz et al. (36) reported that in healthy men, the concentration of blood ionized calcium reaches a peak at 1000 and then decreases progressively during the day to a nadir at 1900, the magnitude of the decrease being ~ 0.26 mg/dl. Similarly, in the present study, when dietary phosphorus was normal the concentration of blood ionized calcium decreased progressively from a morning fasting value of 4.55±0.07 mg/dl to a nadir of 4.38±0.07 mg/dl at 1600 (Fig. 2). When dietary phosphorus was manipulated for 10 d, the morning fasting concentration of ionized calcium did not change significantly, but the levels after breakfast were lower with phosphorus supplementation than with normal dietary phosphorus ($P < 0.05$). The changes induced in the daytime mean blood concentration of ionized calcium varied directly with the changes induced in the serum concentration of 1,25-$(OH)_2$D ($R = 0.49$, $P < 0.05$). During the first 24 h of phosphorus restriction, blood ionized calcium did not change significantly (data not shown).

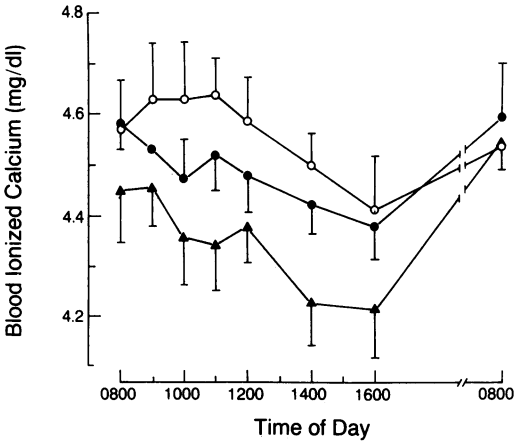

Figure 2. Effect of changes in dietary phosphorus on the concentration of blood ionized calcium in healthy men. Blood was drawn at the times depicted, after subjects had received the normal (●), restricted (○), and supplemented (▲) intakes of phosphorus, each for 9 or 10 d. When phosphorus was supplemented, the values of ionized calcium during the day were significantly lower than the values when phosphorus was normal ($P < 0.05$).

Serum concentration, PR, and MCR of 1,25-$(OH)_2$D. As we previously reported (25), when dietary phosphorus was restricted for 10 d, the serum concentration of 1,25-$(OH)_2$D increased in each subject, and for the group by 80%, from 38 to 68 pg/ml. The calculated PR of 1,25-$(OH)_2$D doubled from 1.8 to 3.8 μg/d; there was no change in its MCR. When phosphorus was supplemented for 10 d, the serum concentration of 1,25-$(OH)_2$D decreased to 27 pg/ml, a value 30% lower than that when dietary phosphorus was normal. The PR decreased by 26% to 1.3 μg/d, and the MCR did not change significantly. In the present study, we found that the changes induced in the PR of 1,25-$(OH)_2$D by manipulation of dietary phosphorus varied inversely and significantly with the changes induced in the 24-h mean serum concentration of phosphorus ($R = -0.88$, $P < 0.001$, Fig. 3). A similar relationship was observed between changes in the serum concentration of 1,25-$(OH)_2$D and changes in the 24-h mean serum level of phosphorus ($R = -0.93$, $P < 0.001$), and between the absolute serum levels of 1,25-$(OH)_2$D and the 24-h mean levels of phosphorus ($R = -0.90$, $P < 0.001$). Since fasting serum levels of phosphorus did not change when dietary phosphorus was supplemented, it is apparent that there is no relationship between fasting levels of phosphorus and either the PR or serum level of 1,25-$(OH)_2$D with phosphorus supplementation. The fasting serum concentrations of 25-OHD, mid-region iPTH, and intact iPTH were normal and did not differ significantly from each other on the three intakes of phosphorus (25).

Urinary phosphorus, calcium, and cAMP. When dietary phosphorus was either normal or supplemented, the total and fractional urinary excretion rates of phosphorus reached a nadir at 0900, and increased progressively during the day to a peak at 1900 (Fig. 4), as we and others have previously described (33, 34, 37, 48–50). The rate of phosphorus excretion (micrograms per minute) varied directly and significantly with the serum concentration ($R = 0.61$, $P < 0.02$) and the filtered load ($R = 0.71$, $P < 0.005$) of phosphorus. When dietary phosphorus was restricted for one day, urinary excretion rate of

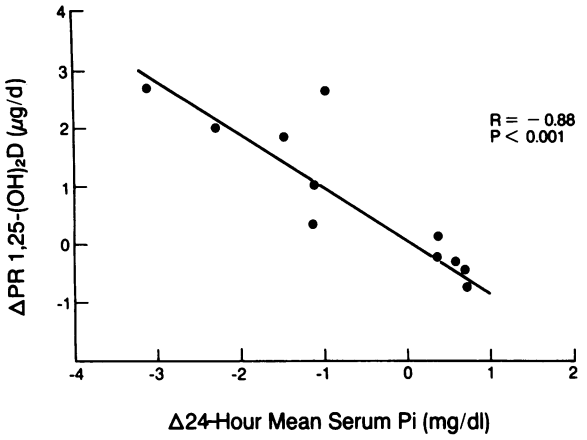

Figure 3. The changes induced in the PR of 1,25-$(OH)_2D$ from control (normal dietary phosphorus) as a function of the changes induced in the 24-h mean serum concentration of phosphorus (Pi) from control, when dietary phosphorus was restricted and then supplemented, each for 10 d. The relationship is inverse and highly significant.

phosphorus decreased immediately and substantially; at 1100, 2 h after initiating phosphorus restriction, the value was half that when dietary phosphorus was normal. Phosphorus excretion remained greatly decreased throughout the day (Fig. 4).

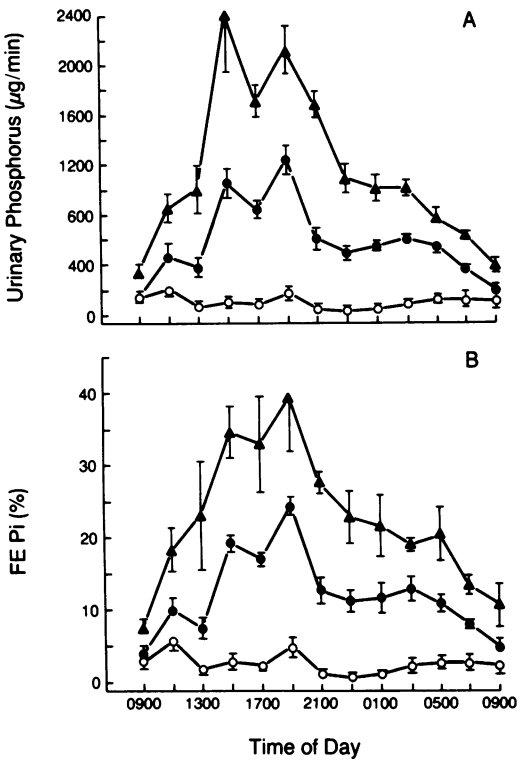

Figure 4. Effect of changes in dietary phosphorus on the total (*A*) and fractional (*B*) urinary excretion rates of phosphorus (Pi) in healthy men. Urine was obtained by voluntary voiding at 2-h intervals for 26 h beginning at 0700, after dietary phosphorus had been normal (●) for 9 d, and after it was restricted (○) for 1 d, and supplemented (▲) for 10 d.

After 10 d of restriction, urinary phosphorus was negligible (< 10 mg/d), and there was no detectable diurnal rhythm in its excretion rate (data not shown). With supplementation of phosphorus, both the absolute and the fractional excretion rates of phosphorus increased substantially, as expected.

When dietary phosphorus was normal, the urinary excretion rate of calcium exhibited a circadian rhythm with a nadir in early morning (0300 to 0500) and a peak in late afternoon (1300 to 1500), a pattern we and others have previously described (34, 37). Phosphorus restriction induced an increase, and phosphorus supplementation a decrease, in the total 24-h excretion rate of phosphorus as expected (25), but induced no change in the general character or phase of its circadian rhythm. The changes in total 24-h urinary excretion of calcium varied directly and significantly with the changes in serum levels of 1,25-$(OH)_2D$ ($R = 0.87$, $P < 0.001$). Phosphorus restriction for 10 d induced a significant 25% decrease in the total 24-h urinary excretion of cAMP; there was no significant change in its excretion with phosphorus supplementation (Table I). The changes in urinary excretion of cAMP varied inversely and significantly with the changes in serum levels of 1,25-$(OH)_2D$ ($R = -0.76$, $P < 0.005$), when data obtained on the three intakes of phosphorus were analyzed as a single set.

Discussion

The results of the present study confirm (34, 36, 37) that in healthy subjects ingesting diets containing "normal" amounts of phosphorus (1–1.5 g/d), the serum concentration of phosphorus exhibits a characteristic circadian rhythm: a nadir in late morning, a rise to a minor peak at ~ 1600 in the afternoon, and a further rise to a major peak just after midnight. Using spectral analysis, we analyzed the time series from each subject individually and found that the variation in serum levels of phosphorus can be described as the sum of sinusoidal functions with periodicities of 24 and 12 h. The magnitude of the variation in phosphorus concentration (peak to trough), as determined from the theoretical curve for each subject, was 1.21±0.2 mg/dl, or 30% of the 24-h mean level, a value identical to that previously reported in healthy men (36), and about one-third of that reported in healthy adolescent male subjects (51).

The present findings demonstrate that restriction of dietary phosphorus induces a near immediate and substantial reduction in the serum concentration of phosphorus. Within 1 h of initiating phosphorus restriction, the concentration of phosphorus decreased significantly and remained decreased throughout the day. After 1 d of restriction, the reduction in serum phosphorus level was already two-thirds of that observed after 10 d of restriction. Phosphorus restriction for 10 d induced a 40% reduction in the 24-h mean serum level of phosphorus, abolished the early afternoon rise in serum phosphorus, as demonstrated by disappearance of the 12-h periodic component of the time series in each subject, and delayed the acrophase by 3 h to 0330. Conversely, phosphorus supplementation for 10 d induced no significant change in the morning fasting serum level of phosphorus, yet did induce a 14% increase in its 24-h mean level. The afternoon rise in serum phosphorus was doubled, such that the afternoon peak in phosphorus level exceeded the nocturnal peak in each subject. The acrophase was thus advanced by 9 h to 1530. These data

demonstrate that in healthy men, changes in dietary phosphorus that induce modest or no change in serum concentration of phosphorus in the morning fasting state, can induce substantial changes in its concentration in the late morning, afternoon, and evening.

Thus, a 24-h circadian rhythm in serum phosphorus concentration occurs in healthy subjects not only when they ingest a small constant amount of fluid throughout the day (33), but also, as demonstrated in the present study, when they ingest a diet made essentially free of absorbable phosphorus by the ingestion of aluminum hydroxide. Indeed in the present study, the amplitude of the circadian rhythm with phosphorus restriction was not different from that with a normal intake of phosphorus. This observation provides evidence that the 24-h rhythm in serum concentration of phosphorus is independent of the intake of phosphorus. The early afternoon rise in serum phosphorus concentration present in subjects ingesting normal amounts of phosphorus, i.e., the 12-h periodic component of the time series, was however abolished by phosphorus restriction and exaggerated by phosphorus supplementation. These observations provide evidence that the afternoon rise in phosphorus concentration is critically determined by dietary phosphorus. Thus, our data would suggest that in healthy men, dietary intake of phosphorus modulates an endogenous 24-h rhythm in serum concentration of phosphorus. The failure of Jubiz et al. (35) to detect a nocturnal peak in serum phosphorus level in healthy subjects receiving neither food nor fluid for 24 h may be a consequence of their having measured serum phosphorus only at 4-h intervals, or perhaps because the rhythm depends upon some amount of caloric intake. On the basis of the present and previous studies (33, 49), it seems likely that the diurnal rhythm in urinary excretion rate of phosphorus is determined in large part by, and not determining of, the rhythm in serum concentration of phosphorus. Accordingly, and given our observations and those of Stanbury (33), it would appear that the 24-h periodicity in serum concentration of phosphorus reflects an endogenous rhythm, possibly caused by a shift of phosphorus between the cellular and the extracellular compartment.

We have reported that changes in the PR of 1,25-$(OH)_2$D account for the 80% increase and the 30% decrease in its serum concentration, which are induced when dietary phosphorus is restricted and supplemented, respectively, for 10 d (25). In the present study, we found that the magnitude of the changes in both the PR and the serum concentration of 1,25-$(OH)_2$D induced by manipulation of dietary phosphorus varied inversely and significantly with the changes induced in the 24-h mean serum concentration of phosphorus ($R = -0.88$ and $R = -0.93$, respectively, $P < 0.001$). A similar relationship obtained between the absolute values of PR [and serum 1,25-$(OH)_2$D] and absolute values of 24-h mean serum phosphorus. That is, the highest production rates of 1,25-$(OH)_2$D were associated with the lowest 24-h mean serum levels of phosphorus, and the lowest production rates of the hormone with the highest levels of phosphorus. Thus, the results of the present study provide support for the hypothesis that in healthy men, changes in the PR and serum concentration of 1,25-$(OH)_2$D induced by manipulation of dietary phosphorus are mediated, at least in part, by changes in the serum concentration of phosphorus, and such changes may not be apparent in the morning fasting state. The changes in serum concentration of phosphorus were associated with, and apparently determining in large part of, parallel changes in urinary excretion of phosphorus. Accordingly, the diet-induced changes in serum levels of phosphorus might effect changes in the production rate of 1,25-$(OH)_2$D by affecting the renal throughput of phosphorus.

The present findings accord with studies in parathyroidectomized chicks and rats (11, 17, 19, 30, 31), in which the activity of 1-hydroxylase and the apparent production of 1,25-$(OH)_2$D varied inversely with the serum concentration of phosphorus. In a recent preliminary report, synthesis of 1,25-$(OH)_2$D by proximal tubules from intact, vitamin D, calcium, and phosphorus-replete rats varied inversely with the phosphorus concentration in the bathing medium (32). The cellular mechanism by which changes in extracellular concentrations of phosphorus induce changes in the activity of renal 1-hydroxylase remains to be determined, and could depend on transcellular flux of phosphorus or some function of its intracellular concentration in the proximal renal tubule. In the rat, the increase in renal production of 1,25-$(OH)_2$D induced by phosphorus restriction appears to be dependent upon the presence of growth hormone (52–54).

We found, as have others (35–37), that the serum concentration of total calcium exhibits periodic variation during the day. It is generally held that the nocturnal decrease in serum concentration of total calcium reflects hemodynamic (dilutional) changes in the serum concentration of albumin (35). In the present study, manipulation of dietary phosphorus induced no significant change in either the fasting or the 24-h mean serum level of total calcium, or in the general character of its rhythm. By contrast, whereas manipulation of dietary phosphorus induced no change in morning fasting blood levels of ionized calcium, levels of ionized calcium after breakfast were significantly lower with phosphorus supplementation, and slightly (but not significantly) higher with phosphorus restriction. The changes in blood ionized calcium might be a consequence of the diet-induced changes in serum concentration of 1,25-$(OH)_2$D. In support of this possibility is the finding that the changes induced in blood levels of ionized calcium varied directly with those induced in serum levels of 1,25-$(OH)_2$D.

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