Effect of Dietary Phosphorus on Circulating Concentrations of 1,25-Dihydroxyvitamin D and Immunoreactive Parathyroid Hormone in Children with Moderate Renal Insufficiency

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Abstract. The hyperparathyroidism character- istic of patients with moderate renal insufficiency could be caused by decreases in the plasma concentration of ionized calcium (Ca^{++}) evoked by: (a) recurring increases in the plasma concentration of inorganic phosphorus that may be detectable only in the post-prandial period; (b) a reversible, phosphorus-mediated suppression of renal 25-hydroxyvitamin D-1α-hydroxylase that decreases the plasma concentration of 1,25-dihydroxyvitamin D (1,25-$(OH)_2D$) enough to decrease both gut absorption and bone resorption of Ca^{++}; (c) both of these. In a group of eight children with moderate renal insufficiency, mean glomerular filtration rate (GFR) 45±4 (SE) ml/min per 1.73 M^2, ages 6–17 yr, we tested these hypotheses by determining the effect of short term (5 d) restriction and supplementation of dietary intake of phosphorus on the plasma concentration of 1,25-$(OH)_2D$, the serum con- centrations of immunoreactive parathyroid hormone (iPTH) and phosphorus, and the fractional renal excretion of phosphorus (FEPi). When dietary phosphorus was nor- mal, 1.2 g/d, the serum concentrations of phosphorus throughout the day were not greater than those of normal control children, and the serum concentrations of car-

boxyl-terminal iPTH (C-iPTH) were greater, 59±9 vs. 17±3 μleq/ml, and unchanging; the serum concentration of intact-iPTH was also greater, 198±14 vs. 119±8 pg/ ml. The plasma concentration of 1,25-$(OH)_2D$ was lower than that of age-matched controls, 27±3 vs. 36±2 pg/ml ($P < 0.01$). When dietary phosphorus was restricted to 0.35 g/d, the plasma concentration of 1,25-$(OH)_2D$ in- creased by 60% to a mean value not different from that of normal controls, while serum concentrations of C- iPTH and intact-iPTH decreased by 25%, the latter con- centration to a mean value not different from that of controls. FEPi decreased from 31 to 9%. When dietary phosphorus was supplemented to 2.4 g/d, the plasma concentration of 1,25-$(OH)_2D$ decreased 32%, while those of C-iPTH and intact-iPTH increased by 131 and 45%, respectively; FEPi increased from 27 to 53%. Plasma con- centrations of 25-hydroxyvitamin D remained normal and unchanged, and GFR did not change when dietary phosphorus was manipulated.

The data demonstrate that in children with moderate renal insufficiency: (a) A normal dietary intake of phos- phorus is attended by a decreased circulating concentra- tion of 1,25-$(OH)_2D$ and an increased concentration of iPTH, but not by recurring increases in the serum con- centration of phosphorus at any time of the day; (b) Di- etary phosphorus is, however, a major determinant of the circulating concentrations of both 1,25-$(OH)_2D$ and iPTH, which vary inversely and directly, respectively, with dietary intake of phosphorus, and increase and decrease, respectively, to normal values when phosphorus is re- stricted for 5 d; (c) Restriction and supplementation of dietary phosphorus induces changes in the serum con- centration of iPTH that correlate strongly but inversely with those induced in the plasma concentration of 1,25-

(OH)$_2$D ($r = -0.88$, $P < 0.001$); and (d) The physiologic responsiveness of the renal tubule to changes in dietary phosphorus is to a substantial extent intact. The data provide support for the second hypothesis stated.

Introduction

1,25-dihydroxyvitamin D$_3$ (1,25-(OH)$_2$D$_3$)[1] is the metabolite of vitamin D currently considered to be the most biologically active with respect to bone resorption and intestinal absorption of calcium and phosphorus (1–4). The synthesis of 1,25-(OH)$_2$D$_3$ from its endogenous precursor, 25-hydroxyvitamin D$_3$, occurs in mitochondria of the proximal renal tubule and is catalyzed by the enzyme, 25-hydroxyvitamin D$_3$-1α-hydroxylase (1-hydroxylase) (5–11). In patients with severe and moderate renal insufficiency, the occurrence of severe (12–23) and moderate (18–24) reductions in the plasma concentration of 1,25-(OH)$_2$D presumably reflects a reduction in the renal synthesis of this hormone (25, 26). In patients or animals with renal insufficiency, such a reduction could reflect a reduced amount of 1-hydroxylase, but could also reflect a reversible suppression of the activity of the remaining enzyme (27–30), even though the activity of this enzyme is normally stimulated by parathyroid hormone (PTH) (31–33), and the circulating level of this hormone is characteristically increased in these patients (21, 22, 34–37). In some circumstances, the activity of 1-hydroxylase and apparent production of 1,25-(OH)$_2$D are not principally determined by PTH, but rather by a suppressive effect of inorganic phosphorus (38–44). In patients with either idiopathic hypercalciuria (43) or primary hyperparathyroidism (44) in whom dietary phosphorus was supplemented, the circulating concentration of 1,25-(OH)$_2$D decreased to normal levels even though the serum concentration of immunoreactive PTH (iPTH) increased, and that of phosphorus remained normal and unchanged (43), or it decreased (44). Accordingly, in patients with moderate renal insufficiency in whom the dietary intake and serum concentration of phosphorus are not increased, but in whom the amount of phosphorus processed by the remaining nephrons is greatly increased, a phosphorus-mediated suppression of the activity of 1-hydroxylase might reduce the renal synthesis and hence the plasma concentration of 1,25-(OH)$_2$D, and thereby contribute to the pathogenesis of the hyperparathyroidism of these patients (21). We report a positive test of this hypothesis in children with moderate renal insufficiency: short term (5 d) restriction and supplementation of dietary phosphorus induced an increase and a further decrease, respectively, in the plasma concentration of 1,25-(OH)$_2$D, and induced a decrease and further increase, respectively, in the serum concentration of iPTH. With restric-

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1. *Abbreviations used in this paper:* C-iPTH, carboxyl-terminal iPTH; 1,25-(OH)$_2$D$_3$, 1,25-dihydroxyvitamin D$_3$; FEPi, fractional renal excretion of phosphorus; GFR, glomerular filtration rate; 25-OHD, 25-hydroxyvitamin D; 1-hydroxylase, 25-hydroxyvitamin D$_3$-1α-hydroxylase; iPTH, immunoreactive parathyroid hormone; PTH, parathyroid hormone.

tion of dietary phosphorus, the circulating concentrations of both 1,25-(OH)$_2$D and iPTH became normal.

Methods

All studies were performed under a protocol approved for use by the Committee on Human Research, University of California at San Francisco. Informed consent was obtained from the parents of all children studied.

Subjects

We studied eight children with moderate renal insufficiency (glomerular filtration rate [GFR] of $\sim$25 to $\sim$50 ml/min per 1.73 M^2) who ranged in age from 6 to 17 yr. Six of the eight children had principally tubulointerstitial renal disease, and two had principally glomerular disease. No child had received phosphorus binding agents, supplements of calcium, or, in seven of the children, supplements of vitamin D or any of its analogues. One child had received oral 1,25-(OH)$_2$D$_3$ (Rocaltrol, Roche Labs), but not during the 4-wk period before the study. The GFR estimated by clearance of iothalamate ranged from 27 to 52 ml/min per 1.73 M^2 in seven of the eight children; in one child the value was 67, and the mean value for all was 45.

We also studied 17 healthy children who ranged in age from 6 to 16 yr. These children had no history of renal, hepatic, or gastrointestinal disease. Four of these children were healthy siblings of the studied patients, one a cousin. Except where otherwise stated, blood was obtained from these subjects in the morning before breakfast.

Procedures

To determine the effect of a normal, then restricted or supplemented dietary intake of phosphorus on the plasma concentration of 1,25-(OH)$_2$D and on the serum concentrations of phosphorus and iPTH, 13 paired studies were performed in the Pediatric Clinical Research Center in the eight children with moderate renal insufficiency. Each child received a constant whole food diet that provided 1,000 mg of calcium and 1,200 mg of phosphorus per day for 3 d (control period). The intake of phosphorus was then either reduced (350 mg/d, seven patients) or increased (2,400 mg/d, six patients) for 5 d (experimental period). Both maneuvers were carried out in five of the eight children; phosphorus restriction was studied first, while phosphorus supplementation was examined in separate studies 2 mo to 2 yr later. Phosphorus was only restricted in two of the eight children and was only supplemented in one. During the reduced intake of phosphorus, aluminum hydroxide was administered to decrease intestinal absorption of phosphorus, and calcium carbonate was administered to maintain constant intake of calcium. During the increased intake of phosphorus, the control diet was supplemented with Fleet's Phosphasoda (129 mg phosphorus per milliliter) given in 3-ml dosages by mouth with each meal. Blood was drawn daily in the morning before breakfast for measurement of serum concentrations of creatinine, calcium, phosphorus, and magnesium. At the end of the control and experimental periods (days 4 and 9), additional blood was obtained for measurement of serum concentrations of iPTH and plasma concentrations of 25-hydroxyvitamin D (25-OHD) and 1,25-(OH)$_2$D. Urine was collected daily in 24-h pools for measurement of concentrations of creatinine, calcium, phosphorus, and magnesium.

To determine whether in the affected children the serum concentrations of calcium, phosphorus, and iPTH and the urinary excretion of phosphorus during the day were different from those of normal children, measurements were made during the day in seven of the eight

children with moderate renal insufficiency (on day 3 of the control period) and in five of the healthy children. The healthy children, also studied at the Pediatric Clinical Research Center, received constant whole food diets that provided 1,000 mg of calcium and 1,200 mg of phosphorus per day for 3–5 d. Meals were offered at 0830, 1215, and 1715 in both groups of subjects. Blood samples were obtained via an indwelling venous catheter before each of the meals, at 30, 60, and 120 min after breakfast and lunch, and at 60 and 120 min after dinner for measurement of serum concentrations of creatinine, calcium, phosphorus, and iPTH. Urine was obtained by voluntary voiding at 2-h intervals from 0600 to 2000 to measure the concentrations of creatinine and phosphorus. Water intake was encouraged during the study.

Laboratory methods

Vitamin D metabolite assay. Approximately 2,500 dpm each of chromatographically purified 25-hydroxy [23,24(n)-^{3}H] vitamin D_3 and 1,25-dihydroxy [23,24(n)-^{3}H] vitamin D_3 (Amersham Corp., Arlington Heights, IL) in 0.02 ml of absolute ethanol were added to each 2-ml plasma sample to determine percentage of recovery. Plasma lipids were extracted twice with diethyl ether (3:1, vol/vol), and the extract was chromatographed on a column (0.7 × 12 cm) of Sephadex LH-20 developed in hexane/chloroform/methanol (9:1:1).

25-OHD. Plasma 25-OHD was quantitated in a manner similar to that described by Eisman et al. (45). The fraction from the LH-20 chromatography that contained 25-OHD was further purified using a Waters Model 6000 high-pressure liquid chromatographic system (Waters Associates, Milford, MA) with two μ-porasil columns in series in a solvent system of hexane/isopropanol (96:4) at a flow rate of 2.0 ml/min. Quantitation of 25-OHD was accomplished by comparison of the ultraviolet absorbance maximum at 254 nm of the sample to that of a series of 25-OHD$_2$ and 25-OHD$_3$ standards. Overall recovery of 25-hydroxy [23,24(n)-^{3}H] vitamin D_3 was 60–65%. The recoveries of 25-OHD$_2$ and 25-OHD$_3$ were assumed to be the same. In normal adult subjects, the plasma concentration of total 25-OHD (25-OHD$_2$ and 25-OHD$_3$) was 24±2 ng/ml (mean±SEM, n = 31).

1,25-(OH)$_2$D. The fraction from the LH-20 chromatography that contained 1,25-(OH)$_2$D was further purified by high-pressure liquid chromatography using two μ-porasil columns in series in a solvent system of hexane/isopropanol (9:1) at a flow rate of 2.0 ml/min. 1,25-(OH)$_2$D was quantitated in duplicate using a competitive protein binding assay modified from those described by Eisman et al. (46) and Shepard et al. (47). Intestinal cytosol binding protein was obtained from normal vitamin D-adequate chicks. Overall recovery of 1,25-(OH)$_2$D was 70–75%. Intraassay and interassay coefficients of variation were 7.7 and 11.6%, respectively. In normal adult subjects, the plasma concentration of 1,25-(OH)$_2$D was 33±3 pg/ml (n = 15).

Serum concentrations of immunoreactive parathyroid hormone were measured by radioimmunoassay in the laboratory of Dr. Sara Arnaud using two antisera: GP-IM, which has high affinity for the intact hormone and its carboxyl-terminal fragments but low affinity for PTH (1–34), and is referred to hereafter as carboxyl-iPTH (C-iPTH), and CH-12M, which has high affinity for the intact hormone, at least a 30-fold lower affinity for PTH (1–34), and no affinity for carboxyl-terminal fragments, which is referred to hereafter as intact-iPTH (48). Serum and urinary concentrations of calcium were measured by atomic absorption spectrophotometry, serum concentrations of phosphorus by a modification of the Fiske-Subbarow method (49), and urinary phosphorus by auto-analyzer.

Data are presented as group means±SEM. The *t* test for paired data was used to compare differences between values obtained in subjects studied during control intake and either low or high intakes of phosphorus. Where the differences observed were not normally distributed, the data were analyzed by means of the Wilcoxon signed rank test. The unpaired *t* test was used for all other group comparisons. Correlation coefficients were calculated by Spearman's rank correlation procedure.

Results

Control conditions. In the eight children with moderate renal insufficiency, the mean plasma concentration of 1,25-(OH)$_2$D was significantly lower than the value in age-matched healthy children (27±3 vs. 36±2 pg/ml, *P* < 0.01) (Table I) when the dietary intake of phosphorus in each child was normal. Such a moderate decrease in circulating level of 1,25-(OH)$_2$D appears to be characteristic of children with moderate renal insufficiency, i.e., GFR of 18–48 (20) or 25–50 (21, 22) ml/min per 1.73 M^2. The plasma concentrations of 25-OHD in the patients and the

Table I. Metabolic and Physiologic Characteristics of Children with Moderate Renal Insufficiency Receiving a Normal Dietary Intake of Phosphorus

	Normal controls	Moderate renal insufficiency
Blood	(17)*	(8)*
1,25-(OH)$_2$D (*pg/ml*)	36±2	27±3‡
25-OHD (*ng/ml*)	22±3	21±3
Carboxyl-iPTH (*μleq/ml*)	17±3	66±10§
Intact-iPTH (*pg/ml*)	119±8	200±17§
Calcium (total) (*mg/dl*)	10.1±0.1	9.8±0.1
Phosphorus (*mg/dl*)	4.9±0.1	4.4±0.2‖
Ccr (ml/min/1.73 M^2)	116±8	45±4§
FEPi (%)	11.0±1.3	30.0±2.6§
Urine		
Phosphorus (mg/kg/24 h)	17.9±1.8	17.9±2.6
Calcium (mg/kg/24 h)	2.8±1.1	0.7±0.2‖
Magnesium (mg/kg/24 h)	3.6±0.4	2.8±0.3

Data are means±SEM. Ccr, creatinine clearance.
* Number of subjects studied. Values for blood concentrations in normal controls were measured in 17 healthy children. Values for Ccr, FEPi, and urinary excretions in controls were calculated from measurements made in the five healthy children studied at the Pediatric Clinical Research Center. All mean values given for the eight children with moderate renal insufficiency were calculated from values measured during the control period when dietary phosphorus was normal. For the five children who were studied twice (phosphorus restriction and supplementation), the values used in the calculation of the mean value were those measured during the control period of their first study, which in each child was a study of phosphorus restriction.
‡ Value vs. normal control value, *P* < 0.01.
§ *P* < 0.001.
‖ *P* < 0.05.

healthy children did not differ significantly. In the children with moderate renal insufficiency, the mean serum concentrations of both C- and intact-iPTH were significantly increased. In both the patients and in the normal children, the serum concentration of phosphorus reached a nadir in the late morning hours and then progressively increased during the early afternoon (Fig. 1). This diurnal pattern is similar to that observed in healthy adult subjects who ingested normal diets (50). In the morning, before breakfast, the mean serum concentration of phosphorus in the patients was significantly lower than that in the five healthy children (4.9 ± 0.3 vs. 5.4 ± 0.3 mg/dl, $P < 0.05$); thereafter, the values in the two groups did not differ significantly. The serum concentration of calcium changed little during the daytime, and the values in the two groups of subjects did not differ significantly. The serum concentration of C-iPTH did not change significantly in the post-prandial period in either the patients or the controls.
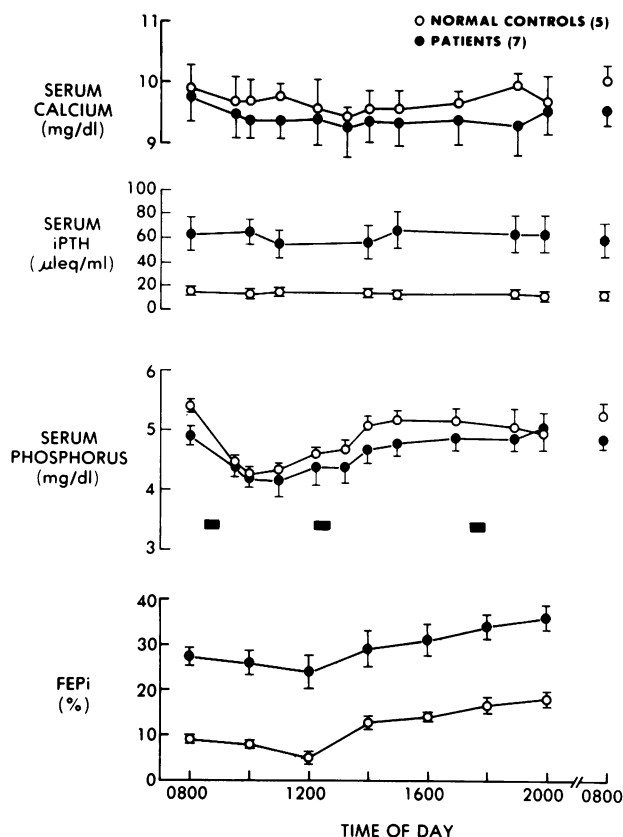

Figure 1. Serum concentrations of calcium, C-iPTH, and phosphorus, and FEPi during the daytime in seven children with moderate renal insufficiency and in five healthy children. Studies were performed after both groups of subjects had received constant whole food diets providing 1,000 mg of calcium and 1,200 mg of phosphorus per day for 2 d. On the day of the study, meals (closed bars) were offered at 0830, 1215 and 1715. The bracketed points depict mean values±SE.

Table II. Metabolic and Physiologic Changes Induced by Restriction and Supplementation of Dietary Phosphorus in Children with Moderate Renal Insufficiency

	Phosphorus restriction (7)*	Phosphorus supplementation (6)
	Change from control value	
Blood		
1,25-(OH)$_2$D (pg/ml)	+16±3‡	−10±2§
25-OHD (ng/ml)	−1±1	−3±1
Carboxyl-iPTH (μleq/ml)	−19±6$^{\|}$	+73±35$^{\|}$¶
Intact-iPTH (pg/ml)	−51±11§	+97±40$^{\|}$¶
Calcium (total) (mg/dl)	−0.1±0.1	−0.2±0.2
Phosphorus (mg/dl)	−0.2±0.1	+0.4±0.3
Magnesium (mg/dl)	−0.2±0.1$^{\|}$	−0.1±0.1
Ccr (ml/min/1.73 M²)	0	2±2
FEPi (%)	−21.4±2.4**	+25.3±3.4**
Urine		
Phosphorus (mg/kg/24 h)	−12.0±2.5‡	+21.6±4.3‡
Calcium (mg/kg/24 h)	+0.6±0.2§	0
Magnesium (mg/kg/24 h)	−0.1±0.4	−0.3±0.3

Subjects received constant whole food diets providing 1,000 mg of calcium and 1,200 mg of phosphorus per day for 3 d. The intake of phosphorus was then either restricted (350 mg/d coupled with administration of aluminum hydroxide) or supplemented (control diet plus oral administration of 1,200 mg phosphorus as Fleet's Phosphasoda) for 5 d. The intake of calcium was maintained constant. Data are expressed as the mean±SEM change from control values.
* Number of subjects studied.
‡ Significant change from control value, $P < 0.005$.
§ $P < 0.01$.
$^{\|}$ $P < 0.05$.
¶ Because the changes induced in iPTH were not normally distributed, the data were analyzed using the Wilcoxon signed rank test.
** $P < 0.001$.

The fractional renal excretion of phosphorus (FEP$_i$) during the daytime was significantly higher in the patients than in the normal controls (Fig. 1), as was the 24-h FEP$_i$ (28 ± 2 vs. $11\pm1\%$, $P < 0.001$) (Table I).

Phosphorus restriction. When the dietary intake of phosphorus was restricted for five days in seven children with moderate renal insufficiency, the plasma concentration of 1,25-(OH)$_2$D increased in each patient from a mean of 26 ± 3 to 42 ± 2 pg/ml ($60\pm11\%$, $P < 0.005$), the latter value being slightly (though not significantly) higher than that in the healthy children who ingested normal amounts of phosphorus (Table II, Fig. 2). Plasma concentrations of 25-OHD did not change significantly. The serum concentration of C-iPTH decreased in six of the seven patients; the mean decrease was 19 ± 6 μleq/ml ($25\pm6\%$, $P < 0.05$). The serum concentration of intact-iPTH decreased
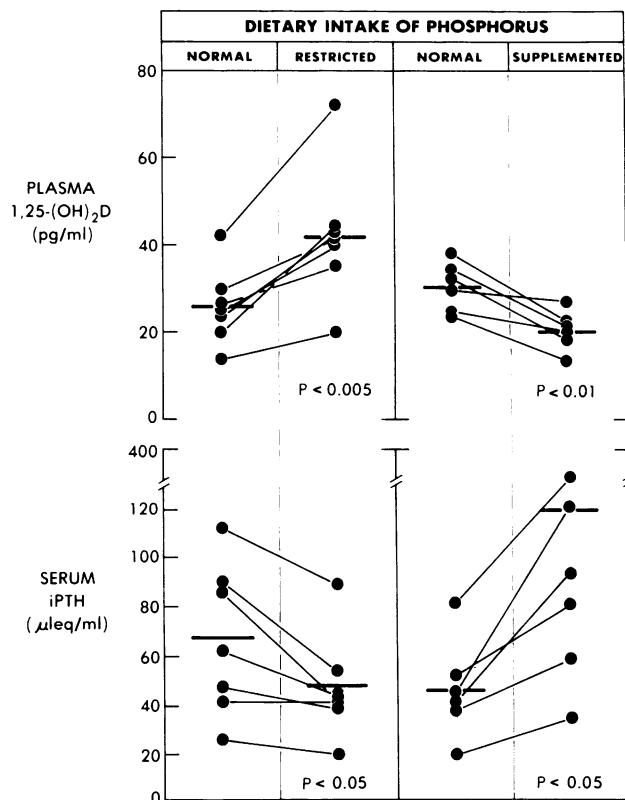
DIETARY INTAKE OF PHOSPHORUS

Figure 2. Effect of 5 d of restriction and of supplementation of dietary phosphorus on the plasma concentration of 1,25-(OH)$_2$D and the serum concentration of C-iPTH in children with moderate renal insufficiency. The dietary intake of phosphorus was normal for 3 d and then either restricted or supplemented for 5 d. The horizontal bars depict means.

in each of the five patients in whom the measurement was made to a mean value not significantly different from that in the controls; the mean decrease was 51 ± 11 pg/ml ($25\pm3\%$, $P < 0.01$) (Table II). The mean fasting serum concentrations of calcium and phosphorus did not change significantly during the period of phosphorus restriction; the serum concentration of magnesium decreased. Both the 24-h total and fractional urinary excretions of phosphorus decreased rapidly and progressively during the period of phosphorus restriction. After 2 d of restriction, both values had decreased by $\sim55\%$ of the control values; after 5 d of restriction, total and fractional excretions of phosphorus had decreased by 75 and 71% of the control values, respectively (Table II). The total urinary excretion of calcium increased significantly within 3 d of the restriction of phosphorus intake. Urinary excretion of magnesium did not change significantly in the four patients in whom the measurement was made.

Phosphorus supplementation. When the dietary intake of phosphorus was supplemented for 5 d, the plasma concentration of 1,25-(OH)$_2$D decreased in five of the six patients studied from a mean of 30 ± 2 to 20 ± 2 pg/ml ($32\pm7\%$, $P < 0.01$) (Table II,

Fig. 2)[2]. Plasma concentrations of 25-OHD did not change significantly. The serum concentrations of both carboxyl- and intact-iPTH increased in each of the patients; the mean increase for C-iPTH was 73 ± 35 μleq/ml ($131\pm39\%$) and for intact-iPTH 97 ± 40 pg/ml ($45\pm17\%$) ($P < 0.05$, respectively, by the Wilcoxon signed rank test). After phosphorus had been supplemented for 2 d, the fasting serum concentration of phosphorus increased by a mean of 1.0 ± 0.5 mg/dl; thereafter it decreased, so that after 5 d of supplementation the mean value was not significantly different from that during the control period (Table II). The mean serum concentrations of calcium and magnesium did not change significantly during the period of phosphorus supplementation. Both the 24-h total and fractional urinary excretions of phosphorus increased progressively during phosphorus supplementation, and the increase for both was significant after 1 d of supplementation. Total urinary excretions of calcium and magnesium did not change significantly.

When the results obtained with high and low dietary intakes of phosphorus were analyzed as a single set, the changes induced in the plasma concentration of 1,25-(OH)$_2$D correlated significantly and inversely with the changes induced in serum concentrations of both carboxyl- and intact-iPTH ($r = -0.88$, $P < 0.001$, Fig. 3). The changes induced in the concentration of 1,25-(OH)$_2$D did not correlate with changes in serum concentrations of calcium, phosphorus, or magnesium, or with changes in total and fractional urinary excretions of phosphorus. The value of endogenous creatinine clearance was not affected by changes in phosphorus intake in any study of any patient (Table II).

Discussion

The present study confirms that in children with moderate renal insufficiency in whom dietary intake of phosphorus is not restricted, the mean plasma concentration of 1,25-(OH)$_2$D is significantly lower than that observed in age-matched controls (20–22), and the serum concentration of iPTH is increased (21, 22, 37). In adult patients with chronic renal insufficiency in whom GFR is below 40–50 ml/min, circulating levels of 1,25-(OH)$_2$D have been found to be decreased and to vary in direct proportion with estimated GFR (17–19, 23, 24). In those adults in whom GFR is ~50 ml/min or greater, the values of 1,25-(OH)$_2$D are normal or even increased (15, 23). In the present study, the observed increase in C-iPTH may reflect in part re-

2. When their phosphorus supplementation studies were performed, 1 and 2 yr, respectively, after their phosphorus restriction studies, two of the five twice-studied patients had become pubescent and were undergoing a somatic growth "spurt". In these two patients the control values of plasma 1,25-(OH)$_2$D (when dietary phosphorus was normal) had increased by 10 and 13 pg/ml, respectively. The higher values attained accounted for a mean control value of 1,25-(OH)$_2$D that was 4 pg/ml higher in the supplementation study than in the restriction study. In healthy children, the plasma concentration of 1,25-(OH)$_2$D increased strikingly during puberty coincident with the peak in growth velocity (50a).
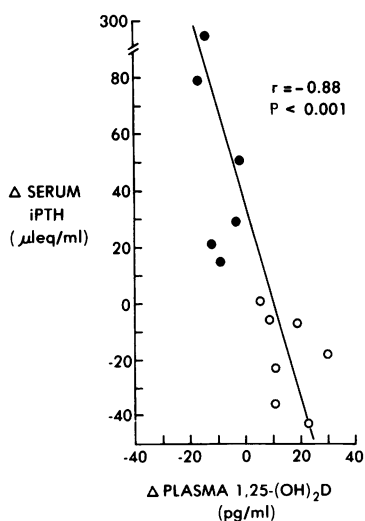
Figure 3. The changes induced from control in the serum concentration of C-iPTH as a function of the changes induced from control in the plasma concentration of 1,25-(OH)$_2$D, when the dietary intake of phosphorus was either restricted (o) or supplemented (•) for 5 d. The changes in plasma concentration of 1,25-(OH)$_2$D correlate inversely with those in serum concentration of iPTH.

duced renal clearance of biologically inactive fragments of PTH (51), whereas the increase in intact-iPTH presumably reflects an increase in biologically active hormone (48). In confirming that restriction of dietary phosphorus can reverse the hyperparathyroidism of patients with moderate renal insufficiency (52), the observations made in the present study, like those made previously in the dog with experimental reduction of renal mass (27, 53–55), provide further support for the formulation that a normal dietary intake of phosphorus can pathogenetically determine the hyperparathyroidism characteristic of chronic renal insufficiency (27, 53–55).

In patients with moderate renal insufficiency, restriction of dietary phosphorus might prevent or ameliorate secondary hyperparathyroidism by preventing an otherwise predictable if transient increase in the serum concentration of phosphorus that increases the release of PTH by reciprocally decreasing the plasma concentration of ionized calcium (56). In such patients, the repeatedly observed and currently confirmed decrease in the morning fasting serum concentration of phosphorus (21, 36, 57, 58) might be a consequence of hyperparathyroidism, which is evoked and possibly amplified by earlier postprandial hyperphosphatemia (59). But in the present study, when the dietary intake of phosphorus was normal in the children with moderate renal insufficiency, the serum concentration of phosphorus was not higher than that of normal control subjects at any time during the day, and the serum concentration of iPTH did not increase after meals in either group of subjects (Fig. 1). Thus, it would be difficult to infer that in the children with moderate renal insufficiency, the extant hyperparathyroidism is either caused by transient increases in the serum concentration of phosphorus or reversed by prevention of such increases.

The present study demonstrates, however, that in the children with moderate renal insufficiency, restriction of dietary phosphorus for 5 d not only decreased the serum concentration of intact-iPTH (and C-iPTH) but also increased the mean plasma

concentration of 1,25-(OH)$_2$D by 60%, the concentrations of both hormones changing to values not significantly different from those of the normal children. Conversely, when dietary phosphorus was supplemented for 5 d, the concentrations of iPTH increased further, and those of 1,25-(OH)$_2$D decreased further by 32%. When dietary phosphorus was restricted or supplemented, the mean plasma concentration of 1,25-(OH)$_2$D ranged from 42 to 20 pg/ml, respectively; the mean serum concentration of C-iPTH ranged from 48 to 119 μleq/ml and that of intact-iPTH from 147 to 297 pg/ml, respectively. These data demonstrate that in children with moderate renal insufficiency, dietary intake of phosphorus is a major determinant of the circulating concentrations of both 1,25-(OH)$_2$D and iPTH, which vary inversely and directly, respectively, with the intake of phosphorus, and increase and decrease, respectively, to normal values when phosphorus is restricted for 5 d.

Thus, with both restriction and supplementation of dietary phosphorus, the circulating concentrations of iPTH vary inversely with those of 1,25-(OH)$_2$D. When the hormonal changes induced in each of the 13 studies are analyzed as a single set, the changes induced in the circulating concentrations of iPTH correlated strongly, but inversely, with those induced in 1,25-(OH)$_2$D ($r = -0.88$, $P < 0.001$, Fig. 3). If the phosphorus-induced changes in circulating iPTH were primary and mediating of changes in the level of 1,25-(OH)$_2$D, one would expect the relationship between these changes to be direct, not inverse. Under normal circumstances, PTH stimulates 1-hydroxylase (31–33) and the production of 1,25-(OH)$_2$D (60). In subjects with intact renal function, increased circulating levels of iPTH are predictably attended by increased levels of 1,25-(OH)$_2$D (14, 61–63), and in patients with primary hyperparathyroidism, the plasma concentration of 1,25-(OH)$_2$D decreased abruptly from greatly increased values to normal values when parathyroid adenomas were surgically removed (64). In normal adult subjects in whom dietary calcium was either restricted or supplemented, the changes induced in the circulating concentration of iPTH correlated directly with those induced in the concentration of 1,25-(OH)$_2$D (65).

We would propose that in the currently studied children with moderate renal insufficiency, manipulation of dietary phosphorus induced changes in the plasma concentration of 1,25-(OH)$_2$D that were primary and mediating of, at least in part, the changes observed in the serum concentration of iPTH. Specifically, by increasing the plasma concentration of 1,25-(OH)$_2$D, phosphorus restriction could have induced an increase in the plasma concentration of ionized calcium by increasing both its intestinal absorption and bone resorption, and thereby could have decreased the release and hence the serum concentration of iPTH. Conversely, by further decreasing the plasma concentration of 1,25-(OH)$_2$D, phosphorus supplementation could have induced the further increase in serum concentration of iPTH. Although plasma-ionized calcium was not measured in the present study, the phosphorus-induced changes in plasma concentration of 1,25-(OH)$_2$D occurred over

a range of values found to correlate directly with intestinal absorption of calcium (19, 61). When hypercalciuria was induced in healthy adult subjects by sodium loading, a 34% increase in serum concentration of 1,25-(OH)₂D was attended by a significant increase in intestinal absorption of calcium (66). That the 60% increase in circulating 1,25-(OH)₂D induced in the present study could have increased intestinal absorption of calcium within 5 d is supported by two observations in normal adult subjects: intestinal absorption of calcium (^{47}Ca) increased significantly when first measured 6–8 d after initiation of presumably physiologic amounts of 1,25-(OH)₂D₃ (0.14 μg/d, per os) (67); and, urinary excretion of calcium increased significantly and to a plateau by the 2nd–5th d of administration of 1,25-(OH)₂D₃ in somewhat greater amounts (0.68 μg/d), suggesting that the maximal effect of this hormone had been realized in this period (68).

The hyperparathyroidism of patients with chronic renal insufficiency may reflect, in part, disordered regulation of secretion of PTH by the plasma concentration of ionized calcium (69, 70). Although it is unclear whether 1,25-(OH)₂D per se can suppress the release of PTH, the increase in plasma concentration of 1,25-(OH)₂D induced in the present studies by phosphorus restriction may have rendered the parathyroid glands more susceptible to suppression by plasma-ionized calcium (71, 72). If so, the suppressive effect of an even minimal increase in plasma-ionized calcium, one that need not be reflected by an increase in the concentration of total calcium, could be amplified by the observed increase in plasma concentration of 1,25-(OH)₂D. Whatever the precise mediating mechanisms, the results of the present study provide support for the hypothesis that in children with moderate renal insufficiency, a normal dietary intake of phosphorus, which is not associated with an increase in serum concentration of phosphorus, entrains a decrease in the plasma concentration of 1,25-(OH)₂D that contributes to the pathogenesis of the hyperparathyroidism characteristic of these patients.

In dogs with experimental chronic renal insufficiency, Kaplan et al. (55) observed that restriction of dietary phosphorus in proportion to the reduction in GFR was attended within 2 d not only by reversal of secondary hyperparathyroidism but also by an increase in the plasma concentration of ionized calcium from a normal to a supernormal level. Over the subsequent 2 wk, the serum concentration of iPTH continued to decrease, while that of ionized calcium remained increased. The serum concentration of phosphorus remained normal and unchanged after phosphorus restriction. The investigators suggested that phosphorus restriction could have caused the persisting hypercalcemia by stimulating the kidney to increase the synthesis and plasma level of 1,25-(OH)₂D, and thereby could have increased the intestinal absorption of calcium; they reported no measurements of plasma 1,25-(OH)₂D. In a preliminary report of adult patients with early renal failure (GFR of 46–70 ml/min), Llach et al. (30) indicated that restriction of dietary phosphorus for 45 d was attended by reversal of hyperparathyroidism,

an increase in both intestinal absorption of calcium and calcemic response to parathyroid extract, and an improvement in bone histology, even though the serum concentration of phosphorus did not change. These investigators too suggested that an increase in plasma concentration of 1,25-(OH)₂D might have mediated the changes induced by phosphorus restriction.

Phosphorus is known to be an important determinant of the renal synthesis of 1,25-(OH)₂D and thereby of its plasma concentration. In parathyroidectomized, vitamin D-deficient chicks and rats, the activity of 1-hydroxylase (33) and the apparent production of 1,25-(OH)₂D (73) varies inversely with the serum concentration of phosphorus. In the intact chick, phosphorus restriction stimulates the activity of 1-hydroxylase (39), and in the intact rat it increases both the plasma concentration (38, 42) and the renal synthesis (74) of 1,25-(OH)₂D. In healthy women, phosphorus restriction induced a 45% increase in the circulating concentration of 1,25-(OH)₂D despite a decrease in the concentration of iPTH (41), and in healthy men it induced an increase in both the serum concentration of 1,25-(OH)₂D and in its rate of production (75). In patients with either idiopathic hypercalciuria (43) or primary hyperparathyroidism (44), supplementation of dietary phosphorus decreased the circulating concentration of 1,25-(OH)₂D despite a significant increase in the concentration of iPTH and no increase in the serum concentration of phosphorus. One group of investigators speculated that the increased intake of phosphorus altered renal synthesis of 1,25-(OH)₂D by altering either the transcellular flux of phosphorus or its concentration in the renal cell (43). Indeed, it has been proposed that renal synthesis of 1,25-(OH)₂D is principally determined by the concentration of inorganic phosphorus in renal cortical cells (73).

The proximal renal tubule is the principal site of both the renal reabsorption of phosphorus (76–78) and the activity of 1-hydroxylase (9–11). In the currently studied children with moderate renal insufficiency, a normal dietary intake of phosphorus was attended by a greatly increased fractional excretion of phosphorus, which reflected an increased rate of phosphorus excretion per nephron, and presumably an increased delivery of phosphorus to more distal segments of the remaining proximal tubules. An increase in intracellular concentration or transepithelial processing of phosphorus in the proximal tubule might suppress the activity of 1-hydroxylase, and thereby restrict the synthesis of 1,25-(OH)₂D, even in the absence of an increased serum level of phosphorus and despite the presence of an increased serum level of iPTH. When the children with moderate renal insufficiency were phosphorus restricted for only 5 d, fractional excretion of phosphorus decreased from 31 to 9%, a value like that found in normal children receiving a normal dietary intake of phosphorus. Thus, in the affected children, even short-term dietary restriction of phosphorus could increase the activity of 1-hydroxylase and production rate of 1,25-(OH)₂D to normal values, through a mechanism that depends upon a reduction in the rate of phosphorus excretion per nephron to normal values.

Acknowledgments

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