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LABORATORY INVESTIGATION

Factors in the development of secondary hyperparathyroidism during graded renal failure in the rat

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Factors in the development of secondary hyperparathyroidism during graded renal failure in the rat. Secondary hyperparathyroidism (2° HPT) develops as a result of renal failure. Hypocalcemia, phosphorus retention, calcitriol deficiency and skeletal resistance to the calcemic action of parathyroid hormone (PTH) are closely interrelated pathogenic factors important for the development of 2° HPT in renal failure. Since previous studies have mainly focused on advanced renal failure, only limited data are available in early renal failure. The goal of the present study was to evaluate how alterations in the dietary calcium and phosphorus composition affect the factors known to contribute to the genesis of 2° HPT in early and more advanced renal failure. To achieve this goal, graded differences in renal function were surgically induced in 453 rats while the dietary content of calcium and phosphorus was varied. Three different diets were used: (1) a high phosphorus diet (HPD), to induce phosphorus retention and stimulate 2° HPT; (2) a high calcium diet (HCaD), to inhibit calcitriol synthesis; and (3) a moderate calcium-moderate phosphorus diet (MCaPD), to separate the effects of high dietary phosphorus and calcium. Based on the serum creatinine (S_{Cr}) concentration rats were assigned to one of four different groups: (1) normal renal function ($S_{Cr} \leq 0.3$ mg/dl); (2) mild renal failure (S_{Cr} 0.4 to 0.6 mg/dl); (3) moderate renal failure (S_{Cr} 0.7 to 0.8 mg/dl); or (4) advanced renal failure ($S_{Cr} \geq 0.9$ mg/dl). As the severity of renal failure increased, progressive 2° HPT developed in each of the dietary groups. In the HPD group, the increase in PTH in normals from 47 ± 2 to 135 ± 14 pg/ml in mild renal failure ($P < 0.001$) was associated with hyperphosphatemia, a decrease in calcitriol and a decreased calcemic response to PTH. In the HCaD group, the increase in PTH in normals from 42 ± 2 to 74 ± 4 pg/ml in mild renal failure ($P < 0.001$) was associated with a decrease in calcitriol. In the MCaPD group, 2° HPT developed (40 ± 2 vs. 70 ± 4 pg/ml, normals vs. mild renal failure, $P < 0.001$) despite normal serum calcium, phosphorus and calcitriol levels; however, a decreased calcemic response to PTH was observed. In advanced renal failure, progressive increases of PTH were observed in all groups, but PTH levels were approximately three-fold greater in the HPD than the HCaD and MCaPD groups (410 ± 24, 114 ± 14 and 138 ± 17 pg/ml, respectively; $P < 0.001$). While serum calcitriol levels were markedly decreased in the HPD and HCaD groups in advanced renal failure, normal calcitriol levels were present in the MCaPD group. In summary, the development and magnitude of 2° HPT in the HPD group could be best explained by the contribution of several additive factors which included: hypocalcemia, phosphorus retention, a calcitriol deficiency and a decreased calcemic response to PTH. In the HCaD group, a calcitriol deficiency appeared to be the most important factor for the development of 2° HPT. In the MCaPD group, a decreased calcemic response to PTH, which may have been due to uremia, was the only factor to which 2° HPT could be attributed.

Considerable effort has been devoted to identifying the factors which are important for the genesis of secondary hyperparathyroidism (2° HPT) in renal failure. These factors include: (1) hypocalcemia [1–4]; (2) phosphorus retention [1, 2, 5]; (3) a deficiency of calcitriol [6, 7]; and (4) skeletal resistance to the calcemic action of parathyroid hormone (PTH) [8–11]. Since these pathogenic factors are closely interrelated, evaluating the independent effect of each factor is often difficult. Hypocalcemia and a calcitriol deficiency have been shown to directly stimulate PTH synthesis and secretion [4, 6, 12, 13]. Both phosphorus retention [10, 14] and a calcitriol deficiency [8] independently contribute to the decreased calcemic response to PTH. Phosphorus retention has also been shown to induce hypocalcemia [10, 15] and decrease calcitriol production [16–18]. Furthermore, Lopez-Hilker et al have even suggested that phosphorus retention may directly stimulate PTH secretion [19]. Finally, since the genesis of 2° HPT is multifactorial, it is likely that much of the reported contradictory data regarding the genesis of 2° HPT in the early stages of renal failure may be due to differences in the combination of factors contributing to the development of 2° HPT.

Despite the large body of information on the pathogenesis of 2° HPT, this information generally lacks an integrative approach; this is especially true in early renal failure in which only limited data are available. During the past several years we have developed an experimental model in the rat in which the degree of surgically induced renal failure can be controlled [10, 11]. Thus, our goal in the present study was to evaluate in different stages of renal failure, how changes in dietary calcium and phosphorus composition affect the factors known to be important for the the development and magnitude of 2° HPT. To accomplish this goal, we performed a series of studies in which the dietary calcium and phosphorus intake was varied in normal rats and in azotemic rats with graded degrees of renal failure. In addition, the calcemic response (CR) to PTH which has not

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been determined in most studies addressing the pathogenesis of 2° HPT was evaluated in rats with early renal failure.

Methods

Male Wistar rats weighing 120 to 160 grams were selected for study. All rats underwent arterial ligation of the left kidney or sham operation. To obtain different degrees of renal function, one to three branches of the renal artery in the hilum were ligated. This was followed one week later by a right nephrectomy or sham operation. During surgical procedures, rats were anesthetized with 50 mg/kg of sodium pentobarbital (Nembutal, Abbott Laboratories, Chicago, Illinois, USA) administered intraperitoneally. Rats were housed in individual cages and pair-fed; each rat received 12 to 15 grams of food daily and was allowed free access to water. Rats ingesting less than 12 grams per day were removed from the study.

Three different diets were used in the 453 rats studied: (1) a high phosphorus diet (HPD; 0.6% calcium, 1.2% phosphorus) to induce phosphorus retention and 2° HPT [10, 11]; (2) a high calcium diet (HCaD; 1.2% calcium, 0.6% phosphorus) to inhibit calcitriol synthesis [20] and to evaluate the effect of high dietary calcium on the development of 2° HPT; and (3) a moderate calcium-moderate phosphorus diet (MCaPD; 0.6% calcium, 0.6% phosphorus) to contrast the effects of high dietary phosphorus and calcium on the development of 2° HPT. All diets were provided by the same supplier (ICN, Cleveland, Ohio, USA) and contained the same vitamin D (100 IU/100 g of diet) and caloric content. In general, the calcium and phosphorus content of our three study diets were in the range stated to be normal for the rat [21–23]. The only exception was the phosphorus content of the high phosphorus diet which was minimally greater than the highest phosphorus content (1%) reported as a normal diet [23]. The study diets were begun immediately after the right nephrectomy or the corresponding sham operation. After 16 to 20 days on the respective diets, blood was obtained after overnight fasting for determination of serum calcium, phosphorus, creatinine, PTH and calcitriol. Based on the serum creatinine concentration, rats were assigned to one of the four following groups: (1) normal renal function; serum creatinine $\leq$ 0.3 mg/dl (N = 136 rats); (2) mild renal failure; serum creatinine 0.4 to 0.6 mg/dl (N = 150 rats); (3) moderate renal failure; serum creatinine 0.7 to 0.8 mg/dl (N = 90 rats); and (4) advanced renal failure; serum creatinine $\geq$0.9 mg/dl (N = 77 rats).

Urinary studies

To assess the relative effect of the different content of dietary calcium and phosphorus, a 24 hour urine for calcium, phosphorus, and creatinine was collected during the last day of the study diet in a select number of rats with normal renal function and with mild renal failure. To determine whether dietary changes induced subtle differences not detected by the measurement of serum PTH, urinary cyclic AMP, a marker of PTH biological activity, was measured in rats with normal renal function. For these urinary studies, rats were housed in individual metabolic cages and urine was collected for 24 hours.

PTH infusion

To evaluate the importance of the CR to PTH in the early development of 2° HPT, a 48-hour PTH infusion was performed in rats with mild renal failure. The use of this method to determine the calcemic response to PTH has been described in detail previously [10, 11]. Briefly, 1-34 rat PTH (Bachem, Torrance, California, USA) from a single lot was administered at a constant rate of 0.11 ug/100 g body wt/hr using a subcutaneously implanted miniosmotic pump (Model 2001, Alza, Palo Alto, California, USA). The CR to PTH was defined as the increase in serum calcium (post-infusion minus basal calcium) after a 48-hour PTH infusion. In our previous studies, rats received a specific study diet for a specified time period and then were changed to a calcium-free, low phosphorus (0.16%) diet during the PTH infusion to evaluate the skeletal response to PTH [10, 11]. However, for the present study we reasoned that the actual response to PTH is modified by the study diet. Thus, although the response to a PTH infusion during the ingestion of the study diet reflects both the skeletal response to PTH and gut absorption of calcium, it more closely resembles the 16 to 20 day period during the induction of 2° HPT in which rats ingested the study diet.

Serum calcium was measured by atomic absorption (Perkin Elmer, Norwalk, Connecticut, USA); serum phosphorus, urinary calcium, and urinary phosphorus with specific kits (Sigma, St. Louis, Missouri, USA); and serum and urinary creatinine with a creatinine analyzer (Beckman, Fullerton, California, USA). Serum PTH was determined using a N-terminal radioimmunoassay (INS, Nichols, San Juan Capistrano, California, USA); this assay has been validated previously for the determination of circulating PTH in the rat [10, 11, 24]. The intraassay and interassay coefficients of variation were 5.8% and 9%, respectively. Serum calcitriol was measured in 171 rats using a radioreceptor assay (INS, Nichols); the intraassay and interassay coefficients of variation were 7.2% and 10.4%, respectively. A previous comparison of this assay with a conventional radioreceptor assay after double column separation showed a significant correlation approaching identity (r = 0.93, N = 32) [10]. Urinary cyclic AMP was measured with a conventional radioimmunoassay kit (Incstar, Stillwater, Minnesota, USA).

Statistics

Comparisons of the serum data, either among groups with the same renal function (different diet) or among groups with the same diet (different renal function) were assessed by ANOVA followed by the Duncan's multiple comparison test. The relation between two variables was assessed by Pearson's linear correlation. Since the urinary data were not normally distributed, comparisons were performed with non-parametric tests, the Kruskal-Wallis followed by the Mann Whitney. P values less than 0.05 were considered significant. Results are expressed as the mean $\pm$ standard error (SE).

Results

As shown in Table 1, in rats with normal renal function, serum calcium was similar in the three dietary groups and serum phosphorus was lower in the HPD than the other two groups ($P < 0.01$). The PTH level was higher in the HPD than the MCaPD group ($P < 0.03$) and urinary cyclic AMP (Table 2) greater in the HPD than both the HCaD and MCaPD groups (73 $\pm$ 4 vs. 57 $\pm$ 4 and 61 $\pm$ 3 pmol/min, respectively, $P < 0.01$). Serum calcitriol was lower in the HCaD than the other two

Table 1. Biochemical data for normal renal function and each stage of renal failure

Serum	HPD	HCaD	MCaPD
Normal renal function			
(N = 136)	N = 66	N = 40	N = 30
Creatinine *mg/dl*	0.25 ± 0.01	0.24 ± 0.01	0.25 ± 0.01
Calcium *mg/dl*	9.9 ± 0.1	9.8 ± 0.1	9.9 ± 0.1
Phosphorus *mg/dl*	7.6 ± 0.2	8.2 ± 0.2[a]	8.3 ± 0.2[a]
PTH[c] *pg/ml*	47 ± 2	42 ± 2	40 ± 2[a]
Calcitriol[d] *pg/ml*	189 ± 11	136 ± 9[a]	185 ± 17[b]
Mild renal failure (N = 150)	N = 56	N = 45	N = 49
Creatinine *mg/dl*	0.50 ± 0.01	0.50 ± 0.01	0.49 ± 0.01
Calcium *mg/dl*	9.7 ± 0.1	9.8 ± 0.1	10.0 ± 0.1
Phosphorus *mg/dl*	8.8 ± 0.3	8.0 ± 0.2[a]	7.7 ± 0.2[a]
PTH *pg/ml*	135 ± 14	74 ± 4[a]	70 ± 4[a]
Calcitriol *pg/ml*	152 ± 11	88 ± 9[a]	227 ± 11[ab]
Moderate renal failure (N = 90)	N = 46	N = 24	N = 20
Creatinine *mg/dl*	0.75 ± 0.01	0.76 ± 0.01	0.73 ± 0.01
Calcium *mg/dl*	8.7 ± 0.2	9.8 ± 0.2[a]	10.0 ± 0.1[a]
Phosphorus *mg/dl*	11.8 ± 0.6	7.8 ± 0.3[a]	7.7 ± 0.3[a]
PTH *pg/ml*	310 ± 24	87 ± 6[a]	107 ± 15[a]
Calcitriol *pg/ml*	103 ± 13	82 ± 10	220 ± 23[ab]
Advanced renal failure (N = 77)	N = 48	N = 16	N = 13
Creatinine *mg/dl*	1.07 ± 0.02	1.06 ± 0.05	1.00 ± 0.04
Calcium *mg/dl*	7.0 ± 0.2	9.9 ± 0.2[a]	9.8 ± 0.2[a]
Phosphorus *mg/dl*	16.4 ± 0.7	7.7 ± 0.4[a]	7.9 ± 0.2[a]
PTH *pg/ml*	410 ± 24	114 ± 14[a]	138 ± 17[a]
Calcitriol *pg/ml*	52 ± 13	55 ± 8	230 ± 35[ab]

Data are: mean ± SE; N = number of rats.
[a] $P < 0.05$ vs. HPD
[b] $P < 0.05$ vs. HCaD
[c] Total PTH measurements = 378; respective numbers in each group starting with normal renal function were 127, 120, 66 and 65.
[d] Total calcitriol measurements = 171; respective numbers in each group starting with normal renal function were 46, 62, 36 and 27.

Table 2. Urinary data

	HPD	HCaD	MCaPD
Normal renal function			
Calcium *mg/24 hr*	0.14 ± 0.03	1.13 ± 0.3[a]	0.28 ± 0.08[b]
Calcium/creatinine	0.019 ± 0.003	0.13 ± 0.11[a]	0.039 ± 0.01[b]
Phosphorus *mg/24 hr*	70 ± 6	4 ± 1[a]	20 ± 2[ab]
Phosphorus/creatinine	11 ± 1.2	0.7 ± 0.1[a]	3.3 ± 0.2[ab]
TRP %	37 ± 8	96 ± 1[a]	83 ± 1[ab]
cAMP *pmol/min*	73 ± 4	57 ± 4[a]	61 ± 3[a]
Creatinine clearance *ml/min*	1.10 ± 0.09	1.18 ± 0.06	1.13 ± 0.07
Mild renal failure			
Calcium *mg/24 hr*	0.17 ± 0.02	0.88 ± 0.3[a]	0.48 ± 0.2
Calcium/creatinine	0.019 ± 0.004	0.063 ± 0.02[a]	0.038 ± 0.01
Phosphorus *mg/24 hr*	77 ± 7	8 ± 1[a]	23 ± 3[ab]
Phosphorus/creatinine	9.7 ± 0.5	1 ± 0.1[a]	3 ± 0.3[ab]
TRP %	13 ± 5	88 ± 4[a]	76 ± 4[ab]
Creatinine clearance *ml/min*	0.80 ± 0.05	0.85 ± 0.08	0.81 ± 0.09

Data are mean ± SE; N = 8 in each group. Abbreviations are: TRP, tubular reabsorption of phosphorus.
[a] $P < 0.05$ vs. HPD
[b] $P < 0.05$ vs. HCaD

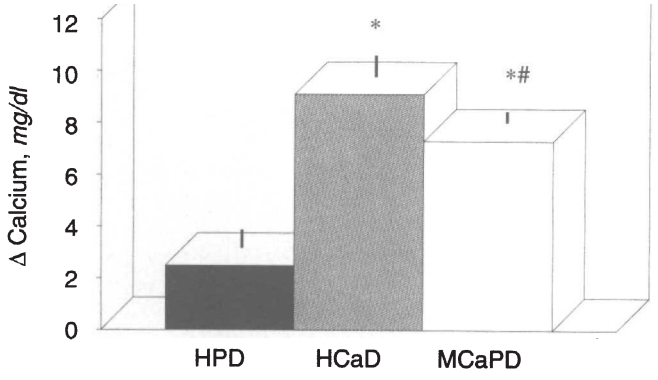

Fig. 1. *Calcemic response to PTH infusion.* Delta calcium is the calcemic response to PTH and represents the difference between the post-PTH infusion and the basal serum calcium. Rats with mild renal failure (serum creatinine 0.4 to 0.6 mg/dl) were infused for 48 hours with 1-34 rat PTH via a subcutaneously implanted miniosmotic pump. Each bar represents the different study diets (HPD, high phosphorus diet, HCaD, high calcium diet, MCaPD, moderate calcium-moderate phosphorus diet). *$P < 0.05$ vs. HPD; #$P < 0.05$ vs. HCaD.

groups ($P < 0.001$). As shown in Table 2, urinary calcium excretion was greater in the HCaD than the HPD group ($P < 0.05$); urinary phosphorus was greater in the HPD than the other two groups ($P < 0.01$) and greater in the MCaPD than the HCaD group ($P < 0.01$). The tubular reabsorption of phosphorus (TRP) was less in the HPD than the other two groups ($P < 0.001$), and lower in the MCaPD than the HCaD group ($P < 0.01$).

In rats with mild renal failure, serum calcium was not different among the three groups (Table 1); however, serum phosphorus was greater in the HPD than the other two groups ($P < 0.001$). Although all three groups developed 2° HPT, the PTH level was approximately twofold higher in the HPD group ($P < 0.001$). The serum calcitriol level was highest in the MCaPD group but was also greater in the HPD than the HCaD group ($P < 0.001$). As shown in Table 2, urinary calcium excretion was greater in the HCaD group than the HPD group ($P < 0.05$). Urinary phosphorus was greater in the HPD than the other two groups ($P < 0.01$) and greater in the MCaPD than the HCaD group ($P < 0.01$). The TRP was less in the HPD than the other two groups ($P < 0.001$), and lower in the MCaPD than the HCaD group ($P < 0.01$).

As shown in Figure 1, the CR to PTH in rats with mild renal failure was greater in the HCaD than in the MCaPD and HPD groups (9.1 ± 0.8, 7.3 ± 0.3 and 2.5 ± 0.6 mg/dl, respectively, $P < 0.001$); furthermore, it was also greater in the MCaPD than in the HPD group ($P < 0.001$). In normal rats maintained on a moderate calcium and phosphorus diet (MCaPD), the CR to PTH was 8.5 ± 0.5 mg/dl. This value was significantly greater than both the MCaPD and HPD groups with mild renal failure.

In moderate renal failure, the HPD group first developed fasting hypocalcemia and hyperphosphatemia increased at this stage of renal failure (Table 1). Serum calcium was less ($P < 0.001$) and serum phosphorus greater ($P < 0.001$) in the HPD than the other two groups. The serum PTH level was elevated in all three groups (Table 1) but was approximately threefold greater in the HPD group ($P < 0.001$). The serum calcitriol level was normal in the MCaPD group and similarly decreased in the HPD and HCaD groups ($P < 0.001$).

In advanced renal failure, the fasting serum calcium was less ($P < 0.001$) and serum phosphorus greater ($P < 0.001$) in the

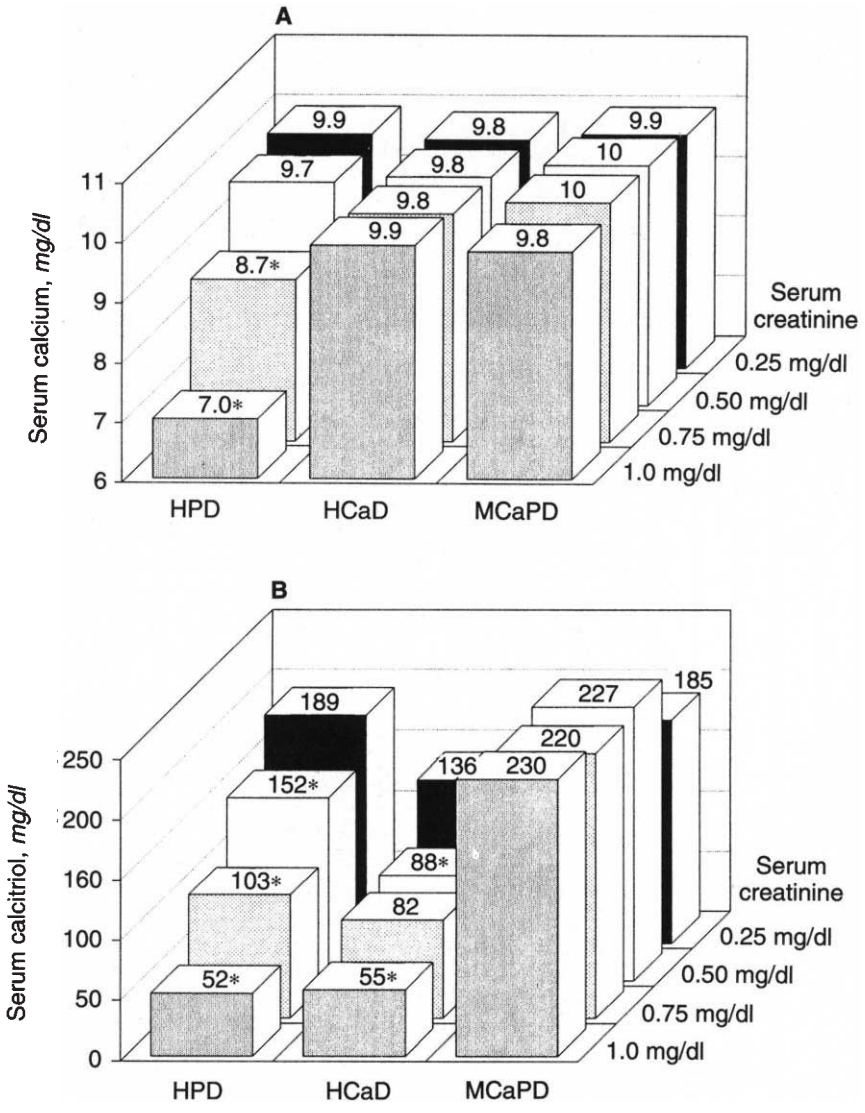

Fig. 2. *Intragroup comparisons of serum calcium and calcitriol.* **A.** The intragroup comparison (same study diet) for serum calcium is shown for each of the four stages of renal function. **B.** The intragroup comparison (same study diet) for serum calcitriol is shown for each of the four stages of renal function. For Figure 2, the lowest serum creatinine is the top row and the highest serum creatinine is the bottom row. The number inserted at the top of each bar is the mean value for the group (HPD, high phosphorus diet; HCaD, high calcium diet; MCaPD, moderate calcium-moderate phosphorus diet). $^*P < 0.05$ vs. preceding lower serum creatinine level.

HPD than the other two groups (Table 1). Although 2° HPT was present in all three groups, PTH levels were three- to four-fold greater in the HPD group than the other two groups ($P < 0.001$). In the MCaPD group, serum calcitriol levels remained in the normal range and were greater than in the HCaD and HPD groups ($P < 0.001$).

Shown in Figures 2 and 3 are the intragroup comparisons for the different dietary groups for the four different levels of renal function. In the HPD group, serum calcium progressively decreased in moderate and advanced renal failure ($P < 0.001$). Serum phosphorus and PTH progressively increased at each stage of renal failure ($P < 0.001$). The PTH level was approximately nine-fold greater in advanced renal failure than in normal rats. Serum calcitriol decreased progressively as renal function deteriorated ($P < 0.001$) and was approximately 25% of normal in advanced renal failure. As shown in Table 3, significant positive correlations were observed between the serum creatinine and serum PTH, phosphorus, calcitriol, and calcium. Moreover, serum PTH correlated with serum phosphorus and inversely correlated with calcitriol and calcium.

The intragroup comparison for the HCaD shows that the serum calcium and phosphorus were similar at every level of renal function (Figs. 2 and 3). Serum PTH increased in mild renal failure and the increment was greater in advanced renal failure ($P < 0.001$). Serum calcitriol decreased in mild renal failure and was lower in advanced renal failure ($P < 0.001$). As shown in Table 3, serum PTH correlated with creatinine and inversely correlated with calcitriol. Serum calcitriol was inversely correlated with serum creatinine.

The intragroup comparison for the MCaPD reveals that although serum calcium, phosphorus and calcitriol did not change at any level of renal function (Figs. 2 and 3), serum PTH progressively increased ($P < 0.001$). As shown in Table 3, PTH only correlated with serum creatinine ($r = 0.74$, $P < 0.001$). Of interest was the finding that when serum creatinine and the CR to PTH were compared in normal rats and rats with mild renal

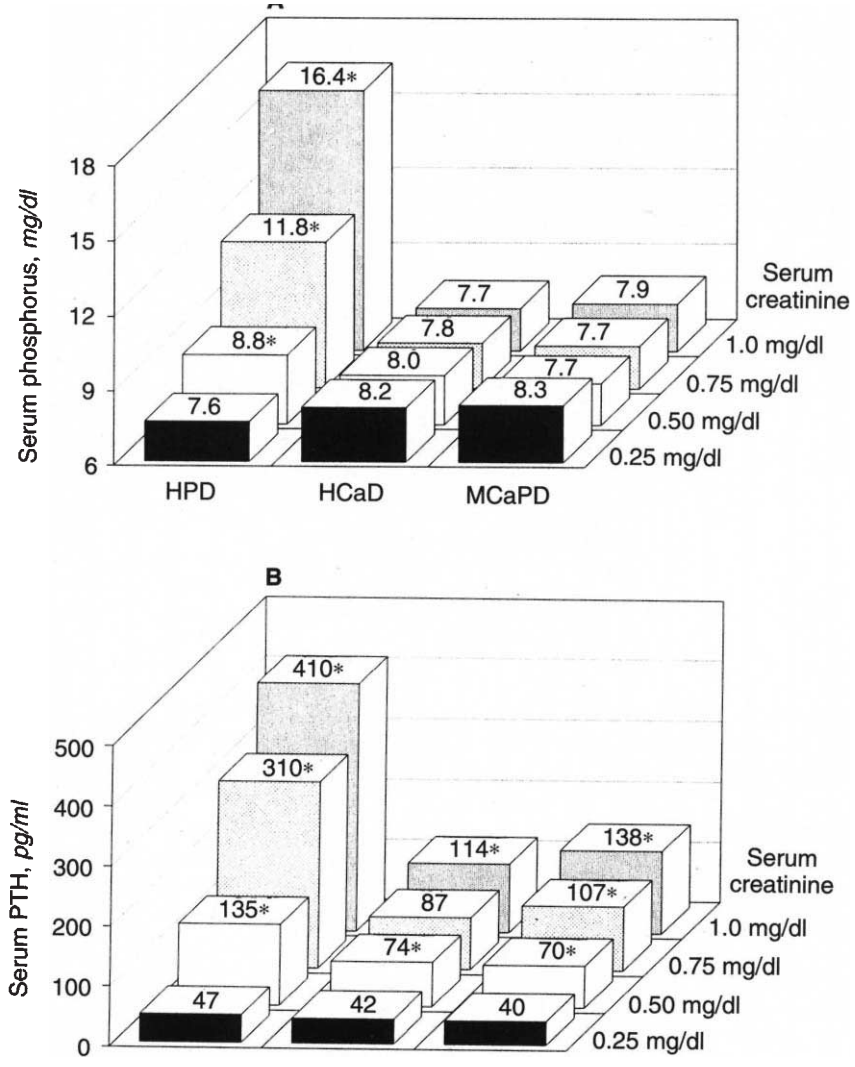

Fig. 3. *Intragroup comparisons of serum phosphorus and PTH.* **A.** The intragroup comparison (same study diet) for serum phosphorus is shown for each of the four stages of renal function. **B.** The intragroup comparison (same study diet) for serum PTH is shown for each of the four stages of renal function. For Figure 3, the lowest serum creatinine is the bottom row and the highest serum creatinine is the top row. The number inserted at the top of each bar is the mean value for the group (HPD, high phosphorus diet; HCaD, high calcium diet; MCaPD, moderate calcium-moderate phosphorus diet). $*P < 0.05$ vs. preceding lower serum creatinine level.

failure, a significant correlation was observed ($r = -0.60$, $P < 0.02$).

The PTH-creatinine regression lines for each diet are shown in Figure 4. The slope of the line was significantly greater in the HPD group than in the MCaPD and HCaD groups ($P < 0.001$; *t*-test with Bonferroni correction).

Discussion

The results of these studies emphasize that the rat is an excellent model to study the development of 2° HPT in renal failure even during the relatively short 16 to 20 day time period used in this study. The relative importance of the pathogenic factors in the development of 2° HPT would appear to depend on the dietary calcium and phosphorus content and the magnitude of renal failure. With the HPD, which induced the most severe 2° HPT at every level of renal function, 2° HPT could be attributed to the cumulative effect of factors which included phosphorus retention, a calcitriol deficiency, a decreased S_{CR} to PTH, and eventually hypocalcemia. For the HCaD, the genesis of 2° HPT could be best attributed to a deficiency of calcitriol. For the MCaPD, a decreased CR to PTH appeared to be the primary factor responsible for the development of 2° HPT. Our results emphasize that even with the same degree of renal failure, the relative contribution of each pathogenic factor to the development of 2° HPT may vary considerably. The current study may also help to explain why in previous studies which have evaluated the development of 2° HPT in early renal failure, serum calcitriol levels have been reported as decreased or normal [25, 26], and sometimes even elevated [7, 27]. Moreover, detailed information on the importance of the CR to PTH in the development of 2° HPT which were lacking in early renal failure are provided in the present study.

High phosphorus diet

The HPD has been used as the traditional method for the induction of 2° HPT in renal failure [10, 11, 24] and induced the most severe 2° HPT in the current study. Factors identified with phosphorus-loading and the induction of 2° HPT in renal failure have included a reduction in calcitriol [28], a decreased CR to PTH [10, 14], and hypocalcemia [1, 2, 10]. Thus, the effect of

Table 3. Pearson's coefficients of correlation (r value) for each diet during the entire range of values from normal renal function to advanced renal failure

	Cr	PTH	CTR	P	Ca
High phosphorus diet (HPD)					
Creatinine Cr, *mg/dl*	—				
PTH *pg/ml*	0.85[a]	—			
Calcitriol CTR, *pg/ml*	−0.78[a]	−0.78[a]	—		
Phosphorus P, *mg/dl*	0.77[a]	0.85[a]	−0.61[a]	—	
Calcium Ca, *mg/dl*	−0.78[a]	−0.90[a]	0.76[a]	−0.82[a]	—
High calcium diet (HCaD)					
Creatinine *mg/dl*	—				
PTH *pg/ml*	0.71[a]	—			
Calcitriol *pg/ml*	−0.61[a]	−0.55[a]	—		
Phosphorus *mg/dl*	0.00	0.12	0.38[b]	—	
Calcium *mg/dl*	0.12	−0.02	0.30	0.18	—
Moderate calcium-moderate phosphorus diet (MCaPD)					
Creatinine *mg/dl*	—				
PTH *pg/ml*	0.74[a]	—			
Calcitriol *pg/ml*	0.25	0.28	—		
Phosphorus *mg/dl*	−0.24	−0.16	0.09	—	
Calcium *mg/dl*	0.16	−0.04	0.08	−0.22	—

[a] $P < 0.001$
[b] $P < 0.05$

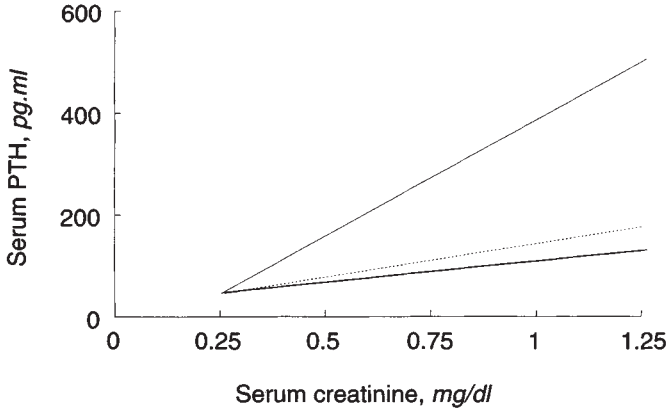

Fig. 4. *The relationship between serum PTH and creatinine.* Serum PTH and creatinine levels are plotted for the entire range of values from normal renal function to advanced renal failure. Each line represents the different study diets (high phosphorus diet, — $y = -66.4 + 454.2\times$, $r = 0.85$; high calcium diet, ▬ $y = 24 + 86.5\times$, $r = 0.71$; moderate calcium-moderate phosphorus diet, ···· $y = 4.2 + 138.1\times$, $r = 0.74$). The least squares regression equation for each diet is presented, and the r value is the correlation coefficient with a P value <0.001 for each group. The slope of the HPD group is significantly greater than the slope of the other two groups ($P < 0.001$).

phosphorus-loading has not been attributed to a direct effect on PTH synthesis and secretion. However, preliminary data from a recent study suggest the possibility that phosphorus may directly stimulate PTH mRNA [29].

In rats with normal renal function, the HPD diet resulted in both higher PTH and urinary cyclic AMP levels, the latter a biological marker of PTH activity. Others have also shown that a high phosphorus diet can induce 2° HPT in normal animals [30]. While hypocalcemia was not observed in the fasting state in the present study, data from Portale et al have shown that phosphorus-loading can induce mild hypocalcemia when serum calcium values are obtained throughout the day [15]. Thus, the possibility that hypocalcemia contributed to the development of 2° HPT should be considered. The normal serum calcitriol level in the presence of phosphorus loading and high PTH levels was likely due to the fact that the inhibitory effect of phosphorus was counteracted by the stimulatory effect of PTH [31]. Furthermore, the decreased serum phosphorus level was likely due to high PTH levels in the fasting state.

During studies in mild renal failure, PTH levels increased by approximately three-fold. Important findings at this level of renal function which likely contributed to the development of 2° HPT were an increase in serum phosphorus, a decrease in serum calcitriol, the likelihood of hypocalcemia during the course of the day [15], and a markedly decreased CR to PTH; the latter reflects an abnormal response to PTH which requires an additional increment in PTH to maintain a specific serum calcium level.

In moderate and advanced renal failure, fasting hypocalcemia was first observed as were further increases in serum phosphorus and decreases in serum calcitriol. In advanced renal failure, serum PTH levels were approximately ten-fold greater than normal. Hypocalcemia has the potential to increase PTH levels by directly stimulating PTH mRNA [3, 4] and also due to its direct stimulation of PTH secretion [32]. Using the same PTH assay as the current study, Uden et al [33] have reported in normal rats that maximal PTH levels induced by hypocalcemia were approximately 2.5 times greater than basal PTH levels. Thus, the ten-fold increase in PTH in the present study must be due to the cumulative effect of factors other than the effect of hypocalcemia on PTH secretion. The presence of a calcitriol deficiency has been shown to increase the transcription of PTH mRNA [4, 6]; at the same time, uremia has been shown to decrease the density of the cytosolic vitamin D receptor [34, 35] and may even decrease the binding of the vitamin D receptor with the vitamin D responsive element of the PTH gene [36].

In the HPD group, significant positive correlations were observed between both PTH and phosphorus and the serum creatinine, and significant inverse correlations between both calcitriol and calcium and the serum creatinine. Moreover, serum PTH correlated with serum phosphorus and inversely correlated with calcitriol and calcium. Our data indicate that the induction of severe 2° HPT by a HPD is through the interaction of additive factors which include hypocalcemia, phosphorus retention, a deficiency of calcitriol, and a decreased CR to PTH. Thus, high dietary phosphorus is an extremely important factor in the genesis of 2° HPT because it inhibits all the counter-regulatory mechanisms which may moderate the severity of 2° HPT.

High calcium diet

As shown by the urinary calcium data in normal rats and in rats with mild renal failure, the HCaD produced calcium-loading. However, a somewhat unexpected finding was that the HCaD also resulted in a decrease in urinary phosphorus excretion despite a similar dietary phosphorus content as the MCaPD; this finding was likely due to some phosphorus binding by calcium in the gut.

The HCaD in normal rats resulted in a significant reduction in calcitriol which was likely due to the known inhibitory effect of a high calcium intake on calcitriol production (20, 31). Despite lower calcitriol levels, PTH did not increase. This was likely

due to the suppressive effect of dietary calcium-loading on PTH synthesis and secretion, thus, the tendency to develop hypercalcemia is counteracted by a reduction in PTH which increases the urinary excretion of calcium. Furthermore, at the molecular level, calcium-loading has been shown to decrease PTH mRNA [37] and most recently, to up-regulate the vitamin D receptor in the parathyroid gland [38].

In mild renal failure, PTH levels increased by almost twice and calcitriol decreased further. Serum calcium and phosphorus were normal as was the CR to PTH. Since both the urinary phosphorus excretion and the TRP reflect mild phosphorus restriction, it is unlikely that phosphorus retention contributed to the development of 2° HPT. Supporting the concept that a calcitriol deficiency is important for the development of 2° HPT is the study by Lopez-Hilker et al in which even hypercalcemia did not prevent the development of 2° HPT in azotemic dogs when a calcitriol deficiency was present [39]. Furthermore, the marked decrease in calcitriol levels in mild renal failure was observed despite elevated PTH levels and moderate dietary phosphorus restriction. Thus, essentially the same PTH level and even greater dietary phosphorus restriction was present in the HCaD than the MCaPD group, and nevertheless the MCaPD group was able to maintain normal calcitriol levels. Consequently, it must be concluded that dietary calcium-loading is an important inhibitor of calcitriol synthesis and this was the only factor which could be shown to induce 2° HPT in the HCaD group. Moreover, the magnitude of the effect of a calcitriol deficiency on the genesis of 2° HPT is difficult to assess because calcium-loading inhibits PTH secretion.

In moderate renal failure, the PTH and calcitriol levels were not different than those observed in mild renal failure, while in advanced renal failure calcitriol decreased and PTH increased further. Among the three dietary groups, the transition from mild to moderate renal failure in the HCaD group was the only instance in which PTH did not increase as renal function decreased. The finding of similar calcitriol levels is consistent with our hypothesis that a calcitriol deficiency is the primary factor in the genesis of 2° HPT in this group.

Moderate calcium and phosphorus diet

In rats receiving a MCaPD, PTH levels almost doubled in mild renal failure, despite normal serum calcium, phosphorus, and calcitriol levels. The only apparent factor contributing to the development of 2° HPT was a decreased CR to PTH. However, it is difficult to be absolutely certain that the reduction in renal function did not result in phosphorus retention. Despite urinary phosphorus excretion was less than one-third of the HPD, it is possible that some degree of phosphorus retention was present. However, against significant phosphorus retention contributing to the development of 2° HPT are several findings that include: (1) the baseline TRP which was 83% on a 0.6% phosphorus diet, considered to be the lower limits of recommended dietary phosphorus [22], was not much lower at 76% in mild renal failure; since both high PTH levels and renal failure independent of PTH increase phosphorus excretion, the 7% decrease in TRP does not suggest significant phosphorus loading; and (2) contrasting findings in rats with advanced renal failure on the MCaPD with those in mild renal failure on the HPD reveals that the MCaPD group was able to maintain a normal serum calcitriol level despite a more severe reduction in renal mass. Conversely, despite a similar three-fold increase in PTH, calcitriol levels were decreased on the HPD even in mild renal failure. Finally, indicating that the decreased CR to PTH was not due to phosphorus-loading in the MCaPD group are our results in a recent study in which we have shown that the CR to PTH in mild renal failure was decreased despite the use of a phosphorus-restricted (0.2%) diet (unpublished observations). Thus, it would appear unlikely that significant phosphorus retention was present on the MCaPD in mild renal failure.

Even though normal serum calcitriol levels were present at all stages of renal function in rats receiving a MCaPD, it is possible that resistance to the suppressive effect of normal calcitriol levels on PTH transcription may have contributed to the development of 2° HPT. While calcitriol resistance has been described in advanced renal failure [40], information is not available on whether calcitriol resistance is present in mild renal failure. The finding of a normal serum calcitriol in all stages of renal failure was somewhat unexpected, but Fukagawa et al have reported similar calcitriol results in 7/8 nephrectomized rats on the same diet as our MCaPD [41]. Thus, it is likely that the stimulus of a potentially mildly restrictive calcium and phosphorus diet together with elevated PTH levels in the presence of normal renal tissue in the remnant kidney was responsible for the normal serum calcitriol level. However, as we have observed, serum calcitriol levels decreased after a longer duration of renal failure despite the same diet and similar PTH levels [42]. Thus, it is likely that intrinsic renal disease develops in the remnant kidney with time and impairs calcitriol production.

Calcemic response to PTH

In the present study, the CR to PTH in rats with mild renal failure was normal with the HCaD, modestly decreased with the MCaPD, and markedly decreased with the HPD. While the CR to PTH was normal in the HCaD group, it must be considered to be due to the calcium content of the diet during the PTH infusion because the CR to PTH for the same rats on a calcium-free diet was lower during the PTH infusion ($9.1 \pm .8$ vs. $6.5 \pm .6$ mg/dl, $P < 0.02$, unpublished data). In the present study, a reduction in the CR to PTH would not appear to contribute to the development of 2° HPT in the HCaD group, but would be relevant for the MCaPD and HPD groups. Factors such as phosphorus retention [10, 14], decreased calcitriol levels [8], and down-regulation of PTH receptors [11, 43] have all been reported to contribute to the decreased CR to PTH in renal failure. In the HPD group, all these factors likely contributed to the decreased CR to PTH. However, in the MCaPD group, a decreased CR to PTH was observed despite a normal serum calcitriol level and minimal if any phosphorus retention. Down-regulation of PTH receptors due to an increase in PTH levels has been stated to be a cause of a decreased CR to PTH in renal failure [43]. However, in our view, this is an unlikely explanation in the MCaPD group since, by definition, down-regulation should only play a role in the development of 2° HPT if PTH is first increased by other means. Moreover, in a recent report in which PTH levels were reduced to the normal range in azotemic rats, we have shown that the CR to PTH remained decreased in azotemic rats [44]. Furthermore, phosphorus retention is also unlikely to be the causative factor since we have observed a decreased CR to PTH in rats with mild renal

failure on a 0.2% phosphorus diet (unpublished observations). Thus, our data in the MCaPD group suggest the possibility that a factor intrinsic to uremia was responsible for the abnormal CR to PTH. Moreover, this is the first time that the CR to PTH has been reported to be decreased in mild renal failure in the presence of normal serum calcitriol levels. The concept that resistance to PTH is an important factor in uremia is also suggested by studies which have shown that high PTH levels are required to maintain a normal bone formation rate in dialysis patients [45, 46]. In a recent study, Sherrard et al questioned whether uremia-induced deficiencies in trophic factors, excesses of growth inhibitors, or receptor or post-receptor defects lead to this resistance [46]. Additional support for the concept that uremia induces hormonal resistance can be derived from other studies in which resistance to other peptide hormones such as insulin and growth hormone has been reported in uremia [47, 48] and even in the early stages of renal failure [49].

In conclusion, results from this study document that the rat is an excellent model to study the development of 2° HPT during the graded reduction in renal function. This comprehensive study in 453 rats has demonstrated that varying the dietary content of calcium and phosphorus can serve to separate individual factors important for the development of 2° HPT and has provided extensive data on the relative importance of factors which contribute to the development of 2° HPT in renal failure. As such, this large body of integrated information can be used as a framework for future investigators to focus on more specific areas in which intact animal studies can be combined with molecular biological techniques to evaluate the interaction of calcitriol, phosphorus, and calcium on the transcription of PTH mRNA and the vitamin D receptor. Furthermore, this study highlights the necessity for studies to evaluate the mechanisms by which uremia affects the development of 2° HPT.

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