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LONG-TERM EFFECTS OF HIGH PHOSPHATE INTAKE ON PARATHYROID HORMONE LEVELS AND BONE METABOLISM

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Evidence has been accumulating over a number of years that phosphate supplements, and possibly dietary phosphate intake, may cause accelerated bone loss. In early studies (Krook & Lowe 1964, Joyce et al. 1971, Draper et al. 1972), predominantly in young animals, high dietary phosphate levels produced frank secondary hyperparathyroidism accompanied by hyperphosphatemia; however, in later studies (Jowsey & Balasubramaniam 1972, Laflamme & Jowsey 1972) in adult animals, a disease resembling osteoporosis was produced and was characterized by increasing bone porosity and normal serum chemistry values. The suggestion has been made that osteoporosis may be a form of low-grade secondary hyperparathyroidism, and in some patients the osteoporosis was found to be associated with serum parathyroid hormone (PTH) levels above normal (Fujita et al. 1973, Riggs et al. 1973).

In a previous study (Laflamme & Jowsey 1972), increased PTH levels and decreased bone mass resulted from oral phosphate supplements. However, the exact sequence of events leading to stimulation of the parathyroid glands was not studied.

The present investigation was designed to find the cause of the bone changes by measuring the effect of a high-phosphate diet on PTH secretion and on bone.

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Table 1. Dietary regimen for control and experimental periods.

Period	Ca : P ratio of diet	Content of diet (g/day)		Time (weeks)
		Ca	P	
1 Normal	1 : 0.7	3.9	2.8	28
2 Control	1 : 0.9	1.1	1.1	35
3 Phosphate-supplemented	1 : 2.2	1.1	2.4	39
4 Phosphate-supplemented	1 : 2.5	1.1	2.7	44
5 Phosphate-supplemented	1 : 2.8	1.1	3.1	48
6 Phosphate-supplemented	1 : 3.1	1.1	3.4	53
7 Phosphate-supplemented	1 : 3.1	1.1	3.4	57

MATERIAL AND METHODS

Six adult female dogs with roentgenographic evidence of closure of the epiphyses were maintained on dog chow (calcium-phosphorus ratio, 1 : 0.7) for 28 weeks. At the beginning of the last month on this diet, fasting-state blood was drawn for serum calcium and phosphorus measurements. The dogs were then given a control diet consisting of Red Heart meat, Wayne dog meal, and bread (calcium-phosphorus ratio, 1 : 0.9) for 7 weeks. This diet, fed twice a day at 0830 and 1600 hours, decreased the daily calcium and phosphorus intakes but did not significantly alter the ratio of these two elements in the diet (Table 1). After the dogs had been on this diet for 4 weeks, they were placed in metabolic cages for 5 days and urine and feces were collected. Fasting-state blood was drawn for serum calcium and phosphorus determinations every month.

Two weeks later, while the same diet was being fed, blood was drawn for a feed sequence. The samples were obtained in the fasting state and ½, 1, 2, 4, 6, and 7½ hours after the morning meal, and serum was analyzed for both total and ionized calcium, phosphorus, immunoreactive PTH (iPTH), total protein, and pH. The dogs then were given oral phosphate supplements in the form of Hyper-Phos-K* in increasing amounts for 5 months. At the final level of phosphate supplementation, the calcium-phosphorus ratio was 1 : 3.1. Fasting-state blood was drawn for serum calcium and phosphorus determinations at the end of each month on the phosphate-supplemented diets, on the day before the supplement was increased. At the end of 2½ and 5 months on the supplemented diets, the 5-day balance studies were repeated. On the last day of the experiment, the feed sequence was repeated and a bone biopsy specimen was taken from the right ulna. Control ulna samples taken from 15 adult dogs were used for comparison. The porosity of the bone was evaluated by counting the number of holes in the cortex on a photographic enlargement (× 42) of the microradiograph; large holes were divided into 2-mm-square spaces, the approximate size of the smallest hole of significant size.

* The Hyper-Phos-K, a gift from Davies Rose Hoyt Pharmaceutical Division of the Kendall Company, was in tablet form; the tablets were ground and added to the diet.

Table 2. Serum calcium, phosphorus, and iPTH values and body weight.

Period	P supplement (g/day)	Serum Ca (mg/dl)	Serum P (mg/dl)	Serum iPTH (μl eq/ml)	Body weight (kg)
1 Normal	0	10.8±0.2	4.6±0.6	–	13.7±2.5
2 Control	0	10.6±0.3	4.6±0.5	55.3±14.1	13.6±2.5
3 Phosphate	2.40	10.5±0.3	3.7±0.5*	–	13.6±1.9
4 Phosphate	2.73	10.5±0.2	3.8±0.9	–	13.4±1.8
5 Phosphate	3.06	10.1±0.3†	4.2±1.2	–	12.7±1.8*
6 Phosphate	3.36	10.4±0.2	3.1±1.2	–	12.7±1.8*
7 Phosphate	3.36	9.7±0.3†	3.9±0.6	142.5±53.2†	13.0±1.8

* For difference from control, $P < 0.05$.

† For difference from control, $P < 0.005$.

Student's t test was used to compare all data. In all tables, the values are the mean and one standard deviation of the mean.

RESULTS

The fasting serum calcium values tended to decrease and were significantly below the control values during the fifth and seventh (phosphate-supplement) periods (Table 2). The fasting serum phosphorus levels also tended to decrease but were significantly lower only during period 3. Serum iPTH increased approximately threefold between the beginning and end of the experiment. The balance studies showed a significant increase in urinary phosphorus and a slight increase in urinary calcium (urinary calcium levels in adult dogs are normally very low compared with man) but no change in fecal calcium or phosphorus from the control period to the end of the experiment (Table 3).

Table 3. Fecal and urinary calcium and phosphorus values during balance studies.

	Control (period 2)	PO_4 supplement for 2½ months	PO_4 supplement for 5 months
Urinary Ca (mg/24 h)	3.48±0.65	6.64±3.65	4.46±1.96
Urinary P (mg/24 h)	262.2±32.7	2,197.4±170.7*	2,398.8±409.6*
Fecal Ca (g/24 h)	1.10±0.10	1.20±0.19	0.92±0.086†
Fecal P (g/24 h)	0.63±0.22	0.97±0.22	0.85±0.16

* For difference from first balance study, $P < 0.0005$.

† For difference between second and third balance studies, $P < 0.05$.

Table 4. Serum calcium (total and ionized), phosphorus, iPTH, and protein during feed sequences.

Time	Serum Ca (mg/dl) Total	Ionized	Serum P (mg/dl)	Serum iPTH (μl eq/ml)	Serum protein (g/dl)
			Control period		
0 hour	10.6±0.3	4.36±0.41	4.6±0.5	55.3±14.1	6.10±0.4
2 hours	10.6±0.2	4.48±0.63	4.1±1.0	74.7±24.7	6.10±0.41
4 hours	10.3±0.3*••	4.65±0.05	4.7±1.0	66.7±20.2	6.00±0.45
6 hours	10.2±0.3****	4.58±0.32†	5.6±1.3†	54.8±11.3	5.80±0.43††
7½ hours	10.1±0.2***•	–	5.7±1.0†	–	–
			Phosphate supplement (period 7)		
0 hour	10.4±0.4	4.11±0.06	3.4±0.5*	142.5±53.2*	5.82±0.50
2 hours	10.5±0.4	4.05±0.75	5.5±0.9*†	159.5±58.4*	5.95±0.41
4 hours	10.1±0.1	3.97±0.12****†	7.7±1.1**†††	186.3±70.4*†	5.80±0.42
6 hours	10.1±0.2	3.98±0.09*	9.3±1.5**†††	149.8±76.0*	5.92±0.42
7½ hours	10.1±0.3***	–	9.3±1.7**†††	–	–

• For difference from control value:
*, $P < 0.05$;
**, $P < 0.01$;
***, $P < 0.005$;
****, $P < 0.001$.

† For difference from T=0 value:
†, $P < 0.05$;
††, $P < 0.01$;
†††, $P < 0.001$.

The feed sequence during the control period, on a calcium-phosphorus ratio of 1:0.9, showed a tendency for the total serum calcium to decrease although the ionized calcium value was increased at 6 hours (Tables 4 and 5). Serum phosphorus decreased initially and then increased at 6 and 7½ hours after feeding. There was no significant change in the serum iPTH levels. After 22 weeks of phosphate supplementation, the feed sequence showed a similar serum calcium decrease after feeding and a similar initial decrease in serum phosphorus which, however, was not statistically significant. This was followed by an increase that was more rapid and more marked compared with the control period. There was a significant change in the serum calcium values although the serum phosphorus levels were lower preprandially and then higher postprandially during the phosphate supplement period. The serum ionized calcium de-

Table 5. Immediate preprandial and postprandial serum calcium and phosphorus values.

Time	Serum Ca (mg/dl)	Serum P (mg/dl)
	Control period	
0 hour	10.6±0.3	4.6±0.5
½ hour	10.6±0.4	3.4±0.6**
1 hour	10.5±0.3	3.7±0.8*
1½ hours	10.5±0.2	3.8±0.9
	Phosphate supplement (period 7)	
0 hour	10.4±0.4	3.4±0.5
½ hour	10.5±0.4	3.1±0.4
1 hour	10.5±0.4	4.0±0.5
1½ hours	10.5±0.3	4.9±0.6*

● For difference from T=0 value: *, $P < 0.05$; **, $P < 0.001$.

creased significantly at 4 and 6 hours after feeding. In this period, as a whole, the ionized calcium values were significantly ($P < 0.001$) lower postprandially (mean ± SD, 4.0 ± 0.1 mg/dl) than preprandially (4.50 ± 0.43 mg/dl). The change in ionized calcium was not associated with any significant alteration in the blood pH, although the phosphate supplements tended to produce an increase in pH. The serum iPTH values all were increased compared with control values and, in addition, the values at 4 hours after feeding were increased significantly above the preprandial values. A significant negative correlation was found between the serum ionized calcium and serum phosphorus values ($r = -0.58$; $P < 0.005$).

The porosity of the ulna cortical bone samples showed a significant increase when compared with samples from adult control dogs fed the normal diet (mean ± SD: normal, 14.3 ± 6.4; phosphate supplement, 59.5 ± 26.6; $P < 0.001$).

DISCUSSION

The calcium-phosphorus ratio in the diet of the average western man is at least 1:2 (0.8 g of calcium to 1.6 g of phosphorus). The normal and control diets in this study therefore represent an unusual calcium-phosphorus ratio. The higher calcium content may explain the slight increase in ionized calcium that occurred postprandially; this is dif-

ficult to explain otherwise in view of the decrease in total calcium, increase in phosphorus, and no significant alteration in serum protein. The immediate decrease in serum phosphorus after phosphate loading is probably an expression of PTH action on the renal excretion of phosphorus because this was found in the fasting animal as well as after feeding; this was related to an increase in hormone levels and also was reflected in the increase in porosity of bone. Presumably, the stimulation of PTH production was mediated by the significant decrease in ionized calcium 4 hours postprandially; ionized calcium also tended to be lower in fasting-state serum.

Reiss et al. (1970) have shown clearly, in a short study in man, that oral ingestion of phosphate supplements will decrease serum ionized calcium and increase serum iPTH levels. The data here extend this observation and confirm the postprandial changes; the main contribution lies in the permanent increase in iPTH related to the tendency for the ionized calcium to be low. Since this appeared in the fasting-state serum, it indicates a sustained effect of the phosphate loading. The significant increase in iPTH 4 hours after feeding may denote an increase in responsiveness of the glands to the stimulus.

An increase in urinary calcium as a result of a small constant dosage of PTH has been noted in normal subjects given aluminum hydroxide gel (Amphojel) (Bartter 1973). In our animals, urinary calcium increased early in the experiment when iPTH levels were minimally elevated (Laflamme & Jowsey 1972); terminally, when hormone levels were higher, urinary calcium tended to decrease. It is possible that, at the later time, PTH was affecting renal calcium excretion and causing retention of calcium while the earlier state with hypercalciuria resembled the condition of normocalcemic primary hyperparathyroidism discussed by Bartter (1973). Long-term phosphate supplementation at low levels may therefore produce a physiologic picture similar to that of osteoporosis.

The increased cortical bone resorption producing increased porosity in the adult dogs reflects the increase in PTH secretion, suggesting that in otherwise normal persons a high phosphate intake will predispose toward bone loss. Because meat has a high content of phosphate, persons on a high meat intake may be expected to become osteoporotic. This indeed appears to be true. A study of an exclusively meat-eating race of Eskimos showed a marked loss of bone density compared to a control omnivorous group; in contrast, a group on a diet of eggs, milk, and vegetables, with a relatively high calcium

intake, had a slower bone loss than the omnivorous group (Mazess 1970, Ellis & Ellis 1972). Although iPTH levels were not measured in these studies, a relationship among high phosphorus intake, stimulation of PTH secretion, and accelerated bone loss is a probable cause of their disease.

Some years ago, Wachman & Bernstein (1968) suggested that the acid content of the diet may play a significant role in bone loss in older persons. Because meat has a high "acid ash", the bone loss in the human studies cited could be the result of both the acid and the high phosphate content of the diet. However, in the present animal study, the additional phosphate was administered as a neutral mixture and there was no significant change in blood pH, either postprandially or in the fasting state, suggesting that phosphate supplements alone will cause bone loss.

Osteoporosis is a common disease. The present studies incriminate the high phosphate intake by the average person as contributory toward the disorder and possibly as a major etiologic factor in the development of bone loss.

SUMMARY

Phosphate supplementation or dietary phosphate content may be an important factor in the etiology of bone loss that occurs with increasing age. Previous studies have suggested that large discrepancies in the phosphorus-to-calcium ratio in favor of phosphorus will produce biochemical and pathologic changes characteristic of secondary hyperparathyroidism. Smaller differences produce a state indistinguishable from osteoporosis. The present study in adult female dogs was designed to investigate the effects of a phosphorus-to-calcium ratio higher than 1 on parathyroid hormone (PTH) secretion and bone morphology. After long-term administration of phosphate, PTH levels were found to be minimally but significantly increased and bone loss was increased. Urinary calcium was not changed, while serum calcium tended to decrease. Postprandial measurements after phosphate administration demonstrated a transient decrease in ionized calcium, which was associated with transient hyperphosphatemia and an increase in serum PTH. Long-term administration of phosphate produced a sustained increase in serum PTH. The studies suggest that the bone loss is mediated by small increases in PTH that result from a phosphate-induced decrease in ionized calcium.

REFERENCES

Bartter, F. C. (1973) Bone as a target organ: toward a better definition of osteoporosis. *Perspect. Biol. Med.* **16**, 215–231.

Draper, H. H., Sie, T.-L. & Bergan, J. G. (1972) Osteoporosis in aging rats induced by high phosphorus diets. *J. Nutr.* **102**, 1133–1141.

Ellis, F. R. & Ellis, J. W. (1972) Incidence of osteoporosis in vegetarians and omnivores. *Amer. J. clin. Nutr.* **25**, 555–558.

Fujita, T., Orimo, H., Okano, K. & Yoshikawa, M. (1973) Clinical application of parathyroid hormone radioimmunoassay. In: *Clinical aspects of metabolic bone disease*, pp. 274–279. Frame, B., Parfitt, A. M. & Duncan, H., editors. Excerpta Medica, Amsterdam.

Jowsey, J. & Balasubramaniam, P. (1972) Effect of phosphate supplements on soft-tissue calcification and bone turnover. *Clin. Sci.* **42**, 289–299.

Joyce, J. R., Pierce, K. R., Romane, W. M. & Baker, J. M. (1971) Clinical study of nutritional secondary hyperparathyroidism in horses. *J. Amer. vet. med. Ass.* **158**, 2033–2042.

Krook, L. & Lowe, J. E. (1964) Nutritional secondary hyperparathyroidism in the horse: with a description of the normal equine parathyroid gland. *Path. Vet.* **1** (Suppl.), 1–92.

Laflamme, G. H. & Jowsey, J. (1972) Bone and soft tissue changes with oral phosphate supplements. *J. clin. Invest.* **51**, 2834–2840.

Mazess, R. B. (1970) Bone mineral content in Wainwright Eskimos: preliminary report. *Arctic Anthropol.* **7**, 114–116.

Reiss, E., Canterbury, J. M., Bercovitz, M. A. & Kaplan, E. L. (1970) The role of phosphate in the secretion of parathyroid hormone in man. *J. clin. Invest.* **49**, 2146–2149.

Riggs, B. L., Arnaud, C. D., Jowsey, J., Goldsmith, R. S. & Kelly, P. J. (1973) Parathyroid function in primary osteoporosis. *J. clin. Invest.* **52**, 181–184.

Wachman, A. & Bernstein, D. S. (1968) Diet and osteoporosis. *Lancet* **i**, 958–959.

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