Long-Term Effect of Low Dietary Calcium:Phosphate Ratio on the Skeleton of *Cebus albifrons* Monkeys[1]

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ABSTRACT Wildcaught cinnamon ringtail monkeys, *Cebus albifrons*, were fed diets with Ca:P ratios of 1:4, 1:2.1, 1:0.4, and 1:0.5 for 3 to 88 months. Monkeys fed the diets with Ca:P ratios of 1:4 and 1:2.1 (ratios similar to that of human diets) had minor microscopic changes suggestive of osteoporosis when compared to other species of animals. The changes were not detected by conventional or magnification radiography or by ^{125}I photon absorptiometry. These findings are in striking contrast to studies in other animals where similar diets resulted in significant bone resorption within 6 weeks to 6 months. This study suggests that the non-human primate may be a more appropriate animal model for the investigation of nutritional osteopenia in man in whom bone resorption appears to be a slowly progressive process. In view of our findings, studies using lower animal species must be re-evaluated with respect to the hypothesis that high dietary phosphate is a significant etiologic factor in senile osteoporosis in man. J. Nutr. *107:* 834–839, 1977.

INDEXING KEY WORDS phosphorus · calcium · bone · non-human primate · photon absorptiometry · magnification radiography

Dietary imbalance of calcium and phosphate has been associated with bone disease in a variety of animal species for many years (1, 2). Clinical and experimental nutritional secondary hyperparathyroidism rapidly developed in horses, dogs, and cats fed diets high in phosphate and low in calcium (3–6). Young rats and mice fed diets low in calcium developed osteoporosis, and aging mice fed a high calcium diet had accelerated bone resorption with increased levels of dietary phosphate (7–9). Similarly, aging rats fed a diet with adequate calcium content developed progressively more severe osteoporosis as dietary phosphate was elevated (10). In a recent study, adult dogs fed diets high in phosphate but adequate in calcium developed rapid bone loss resembling osteoporosis whereas young dogs developed severe nutritional secondary hyperparathyroidism characterized by fibrous osteodystrophy (11). In contrast, some groups of human beings (African Bantu) who consume diets considered low in calcium and relatively high in phosphate have not shown evidence of nutritional osteopenia when compared to other groups (Caucasian) considered to consume diets adequate in calcium and adequate or high in phosphate (12).

As the precise role of varied levels of dietary calcium and phosphate in the pathogenesis of bone disease in man remains uncertain, the purpose of this study was to evaluate the long-term effect of diets with varied calcium and phosphate content on the skeleton and serum electrolytes of non-human primates.

MATERIALS AND METHODS

Nineteen wildcaught juvenile cinnamon ringtail monkeys, *Cebus albifrons*, were housed individually in a room with fluorescent light. The monkeys were divided into three experimental groups and fed purified diets varying in calcium and phos-

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TABLE 1

Experimental design

Group	Cebus no.	Sex	Months in experiment			Body wt, initial	Body wt, final
			Mo	From	To		
						kg	kg
1	341-68	M	88	Nov 68	Feb 76[3]	0.95	2.58
	334-68	M	9	Nov 68	Jul 69	1.14	0.96
	332-68	F	11	Nov 68	Sep 69	1.01	0.70
	333-68	F	77	Nov 68	Mar 75	1.05	1.50
	665-68	F	84	Feb 69	Feb 76	1.50	1.70
	664-68	F	84	Feb 69	Feb 76	1.40	1.76
2	336-68	M	88	Nov 68	Feb 76	1.18	2.30
	338-68	M	2	Nov 68	Dec 68	1.22	0.86
	335-68	M	74	Nov 68	Dec 74	1.19	1.88
	339-68	M	82	Nov 68	Aug 75	1.08	1.28
	663-68	F	84	Feb 69	Feb 76	1.60	1.7
	662-68	F	76	Feb 69	Jun 75	1.10	1.13
3	11	F	88	Nov 68	Feb 76	1.54	2.80
	343-68	M	88	Nov 68	Feb 76	1.01	3.00
	344-68	M	8	Nov 68	Jun 69	0.86	1.07
	340-68	M	9	Nov 68	Jul 69	1.00	1.00
	345-68	M	9	Nov 68	Jul 69	1.20	1.12
	8	F	28	Nov 68	May 71	1.54	1.87
	342-68	M	51	Nov 68	Jan 73	1.05	1.95
4	831-70[1]	M	13	Jan 75	Feb 76	1.80	3.10
	62-75[2]	M	12	Feb 75	Feb 76	2.69	2.60
	63-75[2]	M	12	Feb 75	Feb 76	3.10	2.80
	367-75[2]	M	3	Dec 75	Feb 76	2.51	3.00
	368-75[2]	M	3	Dec 75	Feb 76	3.11	3.20
	369-75[2]	M	3	Dec 75	Feb 76	3.09	3.40
	370-75	M	3	Dec 75	Feb 76	3.00	3.40

[1] = ^{125}I photon absorptiometry only. [2] = Domestic. [3] = All monkeys with follow-up period to Feb 76 are alive, all others are dead.

phate content for up to 88 months (table 1). Each day 150 g of diet were supplied to every monkey. A fourth group of seven captive *Cebus albifrons* was added for the last 3 to 13 months of the experimental period (table 1). Groups 1, 2, and 3 were fed a purified basic diet which contained 25% protein, 8% fat, salt and vitamin mixtures, and 50 mg (2000 IU) of cholecalciferol/kg of diet (13). The basic diet was modified to be low in calcium and high in phosphate for group 1, adequate in calcium and high in phosphate for group 2, and adequate in both calcium and phosphate for control group 3 (table 2). Tap water was available ad libitum (Ca of 67 ppm, range 51–101). Group 4 was fed a commercial primate diet [2] ad libitum containing adequate calcium and phosphate, 15.5% protein, 5.2% fat, and 165 mg (6600 IU) of cholecalciferol per kg of diet (table 2).

Serum was collected monthly from all monkeys in groups 1, 2, and 3 and analyzed by automated procedures [3] for calcium, alkaline phosphatase and inorganic phosphate.

Conventional radiographs were taken of arms, legs and skull of monkeys in groups 1, 2, and 3 at 6 to 12 month intervals. Magnification radiographs of arms and legs were made of all surviving monkeys during the last experimental month.[4] The width and bone mineral content of the radius and ulna of all the surviving monkeys were evaluated at 6 month intervals during the last 2 experimental years using ^{125}I photon absorptiometric methods [5] (14).

[2] Purina Monkey Chow, Ralston Purina Company, St. Louis, Missouri 63199.

[3] Technicon methods N-31P for calcium, method N06B for alkaline phosphate, method N-4C for inorganic phosphate. Technicon Autoanalyzer, Technicon Corp., Ardsley, New York.

[4] Griffiths, H. J., Hunt, R., Grindle, T. & Anderson, M. (1976) The use of a primary magnification technique in metabolic bone disease. Submitted to: J. Vet. Radiol. Soc.

[5] Osteodensitometer, Packard Instrument Corp.,

TABLE 2

Diet compositions

Group	Basic diet	KH_2PO_4	$CaCO_3$	$CaHPO_4$	Ca	P	Ca:P	Cholecalciferol
	g	*g*	*g*	*g*	%	%		*μg/kg diet*
1	957.4	35.1	7.5	0	0.30	1.20	1:4	50
2	929.5	38.2	0	32.3	0.95	2.00	1:2.1	50
3	976.3	0	23.7	0	0.95	0.40	1:0.4	50
4	*[1]	*	*	*	0.86	0.47	1:0.5	165

[1] * Not applicable; group was fed a commercial diet.

Bones were labeled in the seventh year with 1 dose of tetracycline (25 mg/kg body weight, intraperitoneally), followed in 14 days by 1 dose of (2,4 *BIS*) N,N′ DI (Carboxymethyl) (Amino Methyl Fluorescein) (25 mg/kg body weight, intraperitoneally). ^{3}H-proline (100–125 μCi/kg body weight, intraperitoneally) was administered with each of the preceding fluorescent labels. Biopsies of the iliac crests were made 2 days after the last bone label was administered, fixed in 70% ethyl alcohol, embedded in methyl methacrylate and sectioned at 5 to 15 μ for evaluation of the distance between fluorescent labels, the distance between ^{3}H-proline labels using stripping film,[6] and the width of the osteoid seams. An osteoid index was calculated using a compensating polar planimeter[7] by determining the total osteoid area of trabecular bone and dividing by the total area of osteoid plus mineralized bone in the trabeculae of the iliac crest biopsies.

Necropsy was performed on 12 monkeys which died or were killed and tissues were fixed in 10% neutral buffered formalin. Vertebral bodies, femurs, tibias and mandibles were decalcified in formic acid and then with the major viscera were embedded in paraffin, sectioned at 5 μ and stained with hematoxylin and eosin for light microscopic evaluation.

Student's *t*-test for unpaired data was used for the statistical comparison of the experimental groups.

RESULTS AND DISCUSSION

The mean serum values of calcium and phosphate in groups 1, 2, and 3 decreased slightly during the second and third experimental years and then plateaued until the end of the experimental period (table 3). Mean serum alkaline phosphatase values in groups 1, 2, and 3 decreased slightly until the fifth year when they also plateaued (table 3). Statistically significant differences in the mean monthly and yearly serum electrolyte values were not detectable between any 2 of the 3 groups at any time.

Monkeys fed the purified control diet or the experimental diets which had low calcium to phosphate ratios, whether deficient (Ca:P of 1:4) or adequate (Ca:P of 1:2.1) in calcium, did not develop significant clinical, radiographic, or histologic evidence of bone disease during an observation period of 7 years. Microscopic changes in bones were similar in groups 1 through 3. Physeal plates were normal with orderly development of primary and secondary spongiosa. Haversian canals were comparable in number and location in groups 1 through 3 but tended to be slightly more variable in size in groups 1 and 2 compared to control group 3. Although the trabeculae of vertebral bodies in several monkeys in groups 1 and 2 tended to be more slender than others, there were no significant differences in vertebrae from groups 1 through 3. Teeth and mandibles in monkeys of groups 1, 2, and 3 were histologically normal. Fibrous osteodystrophy was not observed in any of the monkeys.

The mean osteoid index of trabecular bone in the iliac crest biopsies was similar in monkeys fed the two purified experimental diets (groups 1 and 2) and the

Downers Grove, Illinois.

[6] Kodak Fine Grain Autoradiographic Stripping Plate AR 10, Eastman Kodak Company, Rochester, New York 14650.

[7] Model #620005, Keuffel and Esser Company, Morristown, New Jersey.

TABLE 3

Serum calcium, phosphate, alkaline phosphatase

Group	1969	1970	1971	1972	1973	1974	1975
				Ca			
				mg/100 ml			
1	10.9±0.9[1] (47)	8.8±0.5 (40)	8.5±0.8 (48)	8.2±0.7 (48)	8.1±0.6 (48)	7.8±0.6 (16)	8.7±0.5 (13)
2	10.9±0.8 (40)	8.8±0.4 (50)	8.8±0.6 (60)	8.4±0.6 (60)	8.1±0.7 (60)	8.1±0.4 (19)	8.9±0.7 (11)
3	11.0±1.3 (52)	8.9±0.6 (40)	8.8±0.7 (40)	8.4±0.7 (36)	8.1±0.6 (25)	8.2±0.3 (8)	8.6±0.5 (8)
				PO_4			
				mg/100 ml			
1	9.4±2.5 (47)	5.9±2.2 (48)	4.4±1.1 (48)	4.8±1.2 (48)	4.6±1.2 (49)	4.8±1.2 (16)	4.9±1.4 (13)
2	9.1±2.8 (41)	6.0±1.3 (50)	6.0±2.5 (60)	5.0±1.6 (60)	4.9±1.6 (60)	4.7±1.3 (19)	5.1±1.7 (11)
3	8.3±2.7 (48)	5.7±2.1 (40)	4.5±1.2 (40)	4.2±1.4 (36)	4.6±1.6 (25)	4.2±1.1 (8)	4.6±1.7 (8)
				Alkaline Phosphatase			
				IU/100 ml			
1	94±42 (43)	120±54 (40)	97±44 (48)	70±42 (48)	44±19 (48)	48±27 (16)	51±26 (13)
2	67±21 (39)	83±32 (50)	51±23 (60)	52±41 (60)	52±29 (60)	51±23 (19)	42±20 (11)
3	109±70 (45)	101±49 (40)	87±39 (40)	78±27 (34)	55±13 (25)	54±16 (8)	48±10 (8)

[1] Mean ± SD, (n).

commercial control diet (group 4) (table 4). Monkeys of group 3 which were fed the purified control diet (Ca:P of 1:0.4) had a greater amount of osteoid (osteoid index of 0.24) than did monkeys of group 1 fed the purified low calcium-high phosphate diet (Ca:P of 1:2.1) which had an osteoid index of 0.08. In comparison, a group of 5 *Cebus albifrons* monkeys fed a diet identical to the purified control diet but deficient in vitamin D developed overt osteomalacia with a greater mean osteoid index of 0.40.[8]

Bone mineralization evaluated by fluorescent labeling revealed similarities in groups 1, 3, and 4 with differences ($P = 0.10$) found only between group 1 and 2 (table 5). Differences in osteoid formation evaluated by ^{3}H-proline labeling were not

[8] Unpublished observation of the authors.

TABLE 4

Osteoid index of iliac crest biopsies in March 1975

Group	n[1]	Osteoid index[2] = osteoid area / (osteoid + bone) area
1	4	0.08±0.01
2	4	0.10±0.06
3	2	0.24±0.08[3,4]
4	6	0.10±0.11

[1] n = number of monkeys. [2] Mean ± SD.
[3] Significantly different from Group 1 ($P = 0.01$).
[4] Different from Group 2 ($P = 0.10$).

TABLE 5

Distance between fluorescent labels and between ^{3}H-proline labels in iliac crest biopsies, in March 1975

Group	n[1]	fluorescent[2]	n[1]	^{3}H-proline[2]
		μ		μ
1	4	6.7±1.6	4	18.1± 6.5
2	4	10.5±3.0[3]	4	26.2±11.4
3	2	7.2±0.5	2	27.1± 6.0
4	2	5.8±1.0	1	21.1

[1] n = number of monkeys. [2] Mean ± SD.
[3] Different from group 1 ($P = 0.10$).

TABLE 6

Bone mineral content (BMU)[1] and bone widths determined by ^{125}I photon absorptiometry

Group	May 74	Jan 75	June 75	Feb 76
		Ulna (BMU)		
1	5.28 ±0.56[2] (4)	4.67 ±0.75 (4)	4.95 ±0.64 (3)	5.07 ±0.57 (3)
2	5.64 ±0.45 (4)	4.79 ±0.52 (4)	4.74 ±0.34 (4)	5.23 ±0.44 (2)
3	5.99 ±0.29 (2)	4.90 ±0.17 (2)	4.60 ±0.33 (2)	4.97 ±0.27 (2)
4	—	7.34 (1)	6.54 ±0.90[4] (3)	6.35 ±0.60[5] (7)
		Radius (BMU)		
1	5.28 ±0.53 (4)	5.18 ±0.50 (4)	5.13 ±0.46 (3)	5.46 ±0.02 (3)
2	55.5 ±0.46 (4)	5.32 ±0.48 (4)	5.20 ±0.51 (4)	5.36 ±0.34 (2)
3	5.76 ±0.56 (2)	5.80 ±0.13 (2)	6.16 ±0.28 (2)	5.48 ±0.98 (2)
4	—	7.46 (1)	6.51 ±0.77[4] (3)	6.79 ±0.79[6] (7)
		Ulna width (mm)		
1	5.96 ±0.42 (4)	6.25 ±0.36 (4)	6.45 ±0.89 (3)	6.61 ±0.65 (3)
2	6.29 ±0.83 (4)	6.26 ±0.38 (4)	6.74 ±0.48 (4)	6.51 ±0.93 (2)
3	6.44 ±0.47 (2)	6.57 ±0.00 (2)	6.01 ±0.00 (2)	6.60 ±0.00 (2)
4	—	8.25 (1)	7.46 ±0.60 (3)	7.17 ±0.50 (7)
1	5.59 ±0.24 (4)	6.54 ±0.40 (4)	6.91 ±0.85 (3)	6.54 ±0.28 (3)
2	5.73 ±0.21 (4)	6.41 ±0.45 (4)	7.16 ±0.33 (4)	6.70 ±0.93 (2)
3	5.86 ±0.36 (2)	6.77 ±0.29 (2)	7.34 ±0.29 (2)	6.94 ±0.59 (2)
4	—	7.90 (1)	6.84 ±0.67[4] (3)	7.67 ±0.34[7] (7)

[1] BMU = Bone mineral unit (Radiology 106(3): 561–564, 1973). [2] Mean ±SD, (n). [3] Significantly different from group 2 ($P < 0.05$). [4] Significantly different from group 3 ($P < 0.05$). [5] Significantly different from groups 1, 2, and 3 ($P < 0.05$). [6] Significantly different from groups 1 and 2 ($P < 0.05$). [7] Significantly different from group 1 ($P < 0.05$) and groups 2 and 3 ($P < 0.05$).

found between any groups of monkeys (table 5).

The minimal histologic differences in the bones of monkeys in groups 1 through 3 were reflected in the absence of detectable radiographic differences in long bones, mandible, skull, and vertebrae in any of the groups during the 7 year experimental period. Cortical thickness, trabecular pattern, and bone density were indistinguishable in groups 1 through 3 by conventional radiographic techniques and in groups 1 through 4 by magnification radiography.

^{125}I photon absorptiometry revealed a trend toward decreased mean bone mineral content in the ulna from the initial to the final evaluation period in groups 1 through 4 (table 6). The mean bone mineral content of the radius increased with age in groups 1, 3, and 4 but decreased slightly in group 2 (table 6). Mean width of the ulna and radius increased in all groups during the 1 to 2 year evaluation period. The only significant differences in bone mineral content or bone widths obtained using ^{125}I photon absorptiometric methods were between group 4 monkeys which were fed a commercial control diet and the other groups of monkeys which were fed purified diets (table 6).

The results of this study are in striking contrast to those of studies in other animal species fed diets with similar calcium to phosphate ratios as well as in contrast to those fed diets with even more imbalanced calcium to phosphate ratios. Joyce et al. (15) and Krook and Lowe (3) found that horses developed facial swelling and lameness within 6 months when fed diets high in phosphate whether calcium was deficient or adequate. Morris et al. (4), Rowland et al. (5), and Krook et al. (6) found that young dogs and cats developed nutritional secondary hyperparathyroidism with fibrous osteodystrophy within 6 to 11 weeks when fed all meat diets (Ca:P of 1:20 to 1:50). Jowsey et al. (11) found that adult and growing dogs had significantly increased bone resorption within 5 months when fed diets with adequate calcium but high in phosphate with a Ca:P ratio of 1:3. Relatively high phosphate containing diets (Ca:P of 1:2 to 1:3) have been suggested as one of the factors involved in the etiology of osteoporosis in humans especially in postmenopausal females.

The results of this study clearly indicate that the short and long-term effect of abnormal dietary Ca:P ratios (1:2.1, 1:4) on the skeleton was insignificant in young growing and maturing monkeys. It is of interest that the *Cebus* monkey does not respond in a fashion similar to other animal species. Although unexplained, this observation stresses caution in extrapolating data from studies of nutritional osteodystrophy in lower animals to explain the pathogenesis of bone disease in human

beings. It is known that certain societies may consume diets with Ca:P ratios of 1:2 and 1:3 but there is no evidence that these diets produce osteoporosis. In view of the taxonomic proximity of humans to non-human primates, it is probable that our results in *Cebus* monkeys are more relevant to humans than those reported using lower animals.

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