Bone Histomorphometric Changes due to Differences in Calcium Intake under Metabolic Acidosis in Rats

Jong-Han WON, Satoshi FUKUDA¹⁾, Reeko SATO, and Yoshihisa NAITO

Department of Veterinary Internal Medicine, Faculty of Agriculture, Iwate University, 3-18-8 Ueda, Morioka, Iwate 020 and ¹⁾Division of Radiotoxicology, National Institute of Radiological Sciences, 4-9-1 Anagawa, Inage-ku, Chiba 263, Japan

(Received 2 October 1995/Accepted 24 February 1996)

ABSTRACT. Effects of differences in calcium (Ca) intake on bone metabolism under metabolic acidosis were examined by bone histomorphometry in rats. Rats were divided into 5 diets; low Ca (0.02%) diet (LCD), moderate low Ca (0.3%) diet (LmCD), standard Ca (0.62%) diet (SCD), moderate high Ca (1%) diet (HmCD) and high Ca (3%) diet (HCD). Each diet rats were subdivided into the acidotic group given a 1.8% ammonium chloride solution as drinking water and control group given deionized water. Blood and double labeled bone were collected 30 days later. Arterial blood pH was significantly lower, and plasma ionized Ca level and urinary Ca excretion were higher in the acidotic groups than those in the control groups in all diets. Breaking force in femur and bone volume in tibial proximal metaphysis were significantly lower in the acidotic groups than those in the control groups in LCD, LmCD and SCD. In the acidotic groups, osteoid thickness was significantly higher in LCD than that in LmCD, HmCD and HCD. Mineral apposition rate in the acidotic groups was significantly higher in LCD and LmCD than that in HmCD and HCD. These results suggest that bone mineral loss and bone fragility under metabolic acidosis may be accelerated by high turnover of bone metabolism due to insufficient dietary Ca intake, but can be prevented by adequate supplementation of Ca. — **KEY WORDS:** bone histomorphometry, dietary calcium, metabolic acidosis, rat.

J. Vet. Med. Sci. 58(7): 611–616, 1996

Metabolic acidosis enhances bone resorption through both cell-mediated processes and direct dissolution of minerals [2–4, 10, 13, 14], and increases in the urinary calcium (Ca) excretion [1, 8, 11, 14]. Whereas, intestinal Ca absorption during metabolic acidosis usually does not increase [8, 11]. These negative effects of metabolic acidosis on mineral metabolism may contribute to the development of bone metabolic disorders such as osteopenia and osteomalacia [9, 14, 17, 20]. Though the alkaline agents have been generally administered to the cases with osteomalacia caused by metabolic acidosis [17], the effects of dietary Ca content on bone metabolism under metabolic acidosis have not been fully elucidated. In this study, therefore, the effects of differences in Ca intake on bone metabolism under metabolic acidosis were examined in rats by bone histomorphometry and measurement of biochemical constituents.

MATERIALS AND METHODS

Animals, diets and treatments: Fifty male Wistar rats, 12 weeks of age, weighing 280–340 g, were used in the present study. All rats were born and bred at our laboratory under controlled temperature ($23 \pm 2^\circ\text{C}$), controlled relative humidity ($55 \pm 10\%$) and a 12:12 hr light-dark cycle (lightening from 06:00 to 18:00). Rats were divided into five diet groups; low Ca (0.02%) diet (LCD), moderate low Ca (0.3%) diet (LmCD), standard Ca (0.62%) diet (SCD), moderate high Ca (1%) diet (HmCD), and high Ca (3%) diet (HCD). The SCD was used as the basal diet of other diets. Each diet contained approximately 20.6% crude protein, 363 Kcal, 0.55% phosphorus (P), 0.05% magnesium, 0.22% sodium, 0.45% potassium, 0.45%

chloride, 0.28% sulfur, and 200 I.U. of vitamin D₃/100 g of diet (Funabashi Farm, Japan). Each diet rats (n=10) were subdivided into the acidotic group (n=5) given a 1.8% solution of ammonium chloride [11, 14] and the control group (n=5) given distilled deionized water as drinking water. The rats were given a respective diet and drinking water containing ammonium chloride or not *ad libitum* for 30 days. The rats were individually housed in metabolic cages (250 × 145 × 210 mm, Tokiwa Co., Japan) for last 7 days of experiment.

Diet intake and body weight: Diet intake was measured at 24 hr intervals for last 3 days of experiment. The body weight in each rat was measured at intervals of 5 days throughout the experimental period.

Analysis of blood and urine: Urine sample was collected at 24 hr intervals for last 3 days. Arterial blood was drawn anaerobically with a heparinized syringe from the abdominal aorta under light anesthesia with pentobarbital sodium (30 mg/kg) prior to sacrifice. Arterial blood pH and carbon dioxide tension (pCO₂) were measured by a blood gas analyzer system (Ciba-Corning Diagnostics, U.S.A.) within 30 seconds after blood collection. Bicarbonate (HCO₃⁻) concentration was calculated using the Henderson-Hasselbalch equation with a pK of 6.1 and a solubility coefficient of 0.030 [23]. Plasma 1,25-dihydroxyvitamin D [1,25-(OH)₂D] level was measured by a radioreceptor assay [22]. Plasma ionized Ca level was measured by a flow-through electrode [19]. Plasma total Ca and urinary Ca levels were measured by atomic absorption spectrophotometry [21]. Plasma and urinary P levels were measured by colorimetry [7].

Analysis of femur: The femur breaking force was measured by a three point bending method using a bone

strength measuring apparatus (Maruto Co., Japan). Subsequently, the femur was ashed at 600°C for 24 hr in an electric furnace and then dissolved in nitric acid. The Ca and P levels were measured by the same method as those of the plasma. The femur Ca and P contents were calculated as a percentage of the wet weight, respectively.

Histomorphometric analysis of tibia: For bone double labeling to analyze bone dynamics, rats were injected intraperitoneally with tetracycline hydrochloride (25 mg/kg) and calcein (8 mg/kg) [24] on days 14 and 22, respectively. The undecalcified tibial proximal metaphysis was stained with Villanueva's bone stain solution, dehydrated in ethanol and acetone, and embedded in methylmethacrylate. The blocks were cut longitudinally and ground at a thickness of 10 to 15 μm with grinding glasses. The histomorphometric analysis of the cancellous bone area in the secondary spongiosa of the tibial proximal metaphysis was performed with a semiautomatic image analyzer (Carl Zeiss, Germany) with Osteoplan II software under fluorescence microscopy [16]. The following static and dynamic parameters were measured; bone volume (volume of trabecular bone including osteoid/volume of total bone, %), trabecular separation (distance between edges of trabecular bone, μm), osteoid thickness (average thickness of osteoid seam, μm), mineral apposition rate (mean distance of tetracycline and calcein labels/labeling intervals, $\mu\text{m}/\text{day}$), and bone formation rate (mean label length $\times$ mineral apposition rate/mean trabecular areas, %/year).

Statistical analysis: Statistical differences between acidotic and control groups in the same diet were evaluated by the Student's *t*-test, and differences among five diets in each acidotic or control groups were analyzed by one-way analysis of variance followed by least significant difference procedure using the Statistical Analysis System (SAS Inst., Inc., U.S.A.). Correlations between dietary Ca content and biochemical values in plasma and urine were analyzed by

linear regression analysis. When the data in blood acid-base parameters were not normally distributed, a Smirnov test was used. The data were expressed as mean $\pm$ SE, and statistical significance was taken at the $P < 0.05$ level.

RESULTS

Diet intake and body weight: Diet intake did not differ significantly in the acidotic groups from that in the control groups in all diets (Table 1). Acidotic groups in all diets showed weight loss during 5 to 10 days of beginning of experiment, then gradually increased. Body weight was lower in the acidotic groups than that in the control groups in all diets throughout experimental period. At 30 days of experiment, body weight in the acidotic groups was significantly lower in LCD than that in SCD and HmCD (Fig. 1).

Blood and urine parameters: Arterial blood pH and HCO_3^- concentration were significantly lower in the acidotic groups than those in the control groups in all diets (Table 1). Plasma ionized Ca level was significantly higher in the acidotic groups than that in the control groups in diets except for HCD (Table 2). Urinary Ca excretion was significantly higher in the acidotic groups than that in the control groups in all diets (Table 2). Plasma total Ca, P, $1,25\text{-(OH)}_2\text{D}$ levels and urinary P excretion did not differ significantly in the acidotic groups from those in the control groups in all diets (Table 2). The correlation coefficients between dietary Ca content and biochemical values in plasma and urine are shown in Table 3.

Femur parameters: Femur breaking force was significantly lower in the acidotic groups than that in the control groups in LCD, LmCD and SCD. Femur Ca and P contents were significantly lower in the acidotic group than those in the control group in LmCD (Fig. 2).

Histomorphometric parameters: Static and dynamic

Table 1. Diet intake and arterial blood acid-base status

Diet	Groups	No. of rats	Diet intake* (g/day)	Arterial pH	pCO ₂ (mmHg)	HCO ₃ ⁻ (mmol/l)
LCD	Control	5	14.8 $\pm$ 1.7	7.393 $\pm$ 0.015	39.3 $\pm$ 1.3	23.6 $\pm$ 0.4
	Acidotic	5	13.0 $\pm$ 2.0	7.219 $\pm$ 0.060*	34.8 $\pm$ 2.3	14.0 $\pm$ 1.2*
LmCD	Control	5	16.1 $\pm$ 0.5	7.421 $\pm$ 0.017	38.5 $\pm$ 1.9	24.6 $\pm$ 0.7
	Acidotic	5	15.8 $\pm$ 1.9	7.333 $\pm$ 0.025*	33.4 $\pm$ 4.1	17.3 $\pm$ 1.4*
SCD	Control	5	15.5 $\pm$ 1.0	7.416 $\pm$ 0.013	33.3 $\pm$ 1.3	21.1 $\pm$ 0.9
	Acidotic	5	16.5 $\pm$ 0.7	7.318 $\pm$ 0.032*	33.8 $\pm$ 3.8	17.0 $\pm$ 1.5*
HmCD	Control	5	17.7 $\pm$ 0.8	7.414 $\pm$ 0.020	36.4 $\pm$ 2.2	22.8 $\pm$ 0.7
	Acidotic	5	17.1 $\pm$ 1.4	7.323 $\pm$ 0.022*	36.8 $\pm$ 2.2	18.7 $\pm$ 1.1*
HCD	Control	5	17.7 $\pm$ 0.9	7.423 $\pm$ 0.014	33.7 $\pm$ 1.5	21.7 $\pm$ 1.1
	Acidotic	5	17.1 $\pm$ 0.8	7.338 $\pm$ 0.019*	34.2 $\pm$ 0.9	18.1 $\pm$ 0.4*

LCD: low Ca (0.02%) diet, LmCD: moderate low Ca (0.3%) diet, SCD: standard Ca (0.62%) diet, HmCD: moderate high Ca (1%) diet, HCD: high Ca (3%) diet. pCO₂: carbon dioxide tension, HCO₃⁻: bicarbonate concentration. Values are mean $\pm$ SE. *: Values are mean at days 28, 29 and 30 of study. *: Different from the control group given same diet ($P < 0.05$).

Fig. 1. Changes in body weight. LCD: low Ca (0.02%) diet, LmCD: moderate low Ca (0.3%) diet, SCD: standard Ca (0.62%) diet, HmCD: moderate high Ca (1%) diet, HCD: high Ca (3%) diet. Values are mean $\pm$ SE.

Table 2. Levels of ionized calcium, total calcium, phosphorus and 1,25-dihydroxyvitamin D [1,25-(OH)₂D] in plasma, and calcium and phosphorus in urine

Diet	Groups	Plasma	Plasma		Urine*		Plasma
		ionized calcium (mmol/L)	Total calcium (mg/dl)	Phosphorus (mg/dl)	Calcium (mg/24h)	Phosphorus (mg/24h)	1,25 - (OH) ₂ D (pg/ml)
LCD	Control	1.31 $\pm$ 0.01 ^{a,b}	9.9 $\pm$ 0.1 ^a	6.0 $\pm$ 0.1 ^a	0.07 $\pm$ 0.02 ^a	14.13 $\pm$ 3.88 ^a	113.9 $\pm$ 10.9 ^d
	Acidotic	1.38 $\pm$ 0.01 ^{*c}	9.7 $\pm$ 0.2 ^c	6.0 $\pm$ 0.4 ^c	0.36 $\pm$ 0.11 ^{*c}	17.31 $\pm$ 1.95 ^c	116.8 $\pm$ 8.6 ^x
LmCD	Control	1.29 $\pm$ 0.02 ^a	10.2 $\pm$ 0.1 ^{a,b}	5.9 $\pm$ 0.3 ^a	0.09 $\pm$ 0.03 ^a	11.67 $\pm$ 1.52 ^a	78.7 $\pm$ 8.7 ^c
	Acidotic	1.39 $\pm$ 0.02 ^{*c}	10.1 $\pm$ 0.1 ^z	6.4 $\pm$ 0.2 ^y	0.33 $\pm$ 0.07 ^{*c}	15.85 $\pm$ 1.06 ^c	67.8 $\pm$ 4.6 ^c
SCD	Control	1.30 $\pm$ 0.01 ^a	10.6 $\pm$ 0.2 ^{b,c}	6.2 $\pm$ 0.1 ^a	0.18 $\pm$ 0.06 ^a	8.23 $\pm$ 0.73 ^{a,b}	55.9 $\pm$ 4.6 ^b
	Acidotic	1.39 $\pm$ 0.02 ^{*c}	10.3 $\pm$ 0.2 ^{z,y}	5.9 $\pm$ 0.4 ^y	0.53 $\pm$ 0.12 ^{*c}	8.88 $\pm$ 0.53 ^y	47.7 $\pm$ 6.4 ^{c,y}
HmCD	Control	1.34 $\pm$ 0.01 ^b	10.9 $\pm$ 0.3 ^{c,d}	5.7 $\pm$ 0.5 ^a	0.29 $\pm$ 0.07 ^a	4.94 $\pm$ 0.99 ^{b,c}	31.1 $\pm$ 3.4 ^a
	Acidotic	1.39 $\pm$ 0.02 ^{*c}	10.8 $\pm$ 0.3 ^{y,x}	5.0 $\pm$ 0.5 ^y	0.55 $\pm$ 0.14 ^{*c}	8.22 $\pm$ 1.82 ^y	37.4 $\pm$ 6.2 ^y
HCD	Control	1.41 $\pm$ 0.02 ^c	11.2 $\pm$ 0.2 ^d	3.6 $\pm$ 0.3 ^b	1.86 $\pm$ 0.38 ^b	0.11 $\pm$ 0.05 ^c	41.8 $\pm$ 10.0 ^{a,b}
	Acidotic	1.46 $\pm$ 0.02 ^y	11.3 $\pm$ 0.3 ^x	3.4 $\pm$ 0.2 ^y	4.70 $\pm$ 1.11 ^{*y}	0.10 $\pm$ 0.02 ^x	51.1 $\pm$ 13.7 ^{c,y}

LCD: low Ca (0.02%) diet, LmCD: moderate low Ca (0.3%) diet, SCD: standard Ca (0.62%) diet, HmCD: moderate high Ca (1%) diet, HCD: high Ca (3%) diet. Values are mean $\pm$ SE. *: Values are mean on days 28, 29 and 30 of study. *: Different from the control group given same diet (P<0.05). a-d) Means in the each control column without a common superscript differ (P<0.05). z, y, x) Means in the each acidotic column without a common superscript differ (P<0.05).

Table 3. Correlation coefficients between dietary Ca content and biochemical values in plasma and urine in control and acidotic groups

Parameters	Control group	Acidotic group
Plasma 1,25-(OH) ₂ D ^a	-0.567	-0.603
Plasma ionized calcium	0.804	0.580
Plasma total calcium	0.663	0.747
Plasma phosphorus	-0.811	-0.801
Urine calcium	0.872	0.830
Urine phosphorus	-0.688	-0.869

a) 1,25-dihydroxyvitamin D. All values are significant at P<0.01.

Fig. 2. Effect of metabolic acidosis and/or dietary calcium content on breaking force and contents of calcium and phosphorus in the femur. LCD: low Ca (0.02%) diet, LmCD: moderate low Ca (0.3%) diet, SCD: standard Ca (0.62%) diet, HmCD: moderate high Ca (1%) diet, HCD: high Ca (3%) diet. Values are mean $\pm$ SE.

Fig. 3. Effect of metabolic acidosis and/or dietary calcium content on static and dynamic histomorphometric parameters in the secondary spongiosa of tibial proximal metaphysis. LCD: low Ca (0.02%) diet, LmCD: moderate low Ca (0.3%) diet, SCD: standard Ca (0.62%) diet, HmCD: moderate high Ca (1%) diet, HCD: high Ca (3%) diet. Values are mean $\pm$ SE.

histomorphometric data are shown in Fig. 3. Bone volume was significantly lower but trabecular separation was significantly higher in the acidotic groups than those in the control groups in LCD, LmCD and SCD. In the acidotic groups, osteoid thickness was significantly higher in LCD than that in LmCD, HmCD and HCD. In the acidotic groups, mineral apposition rate was significantly higher in LCD and LmCD than that in HCD and HmCD. Bone formation rate was significantly lower in the acidotic group

than that in the control group in HCD, and tended to be lower in other diets.

DISCUSSION

Metabolic acidosis and alkalosis refer to changes of blood hydrogen ion and HCO_3^- concentration not attributed to changes in blood pCO_2 . Metabolic acid-base status of blood can be attributed to the inorganic cations and anions of the

blood and cells; which are ultimately derived from the diet [23]. Therefore, metabolic acidosis can be induced by the addition of anion such as chloride to drinking water and diet [1, 5, 6, 8, 11, 14, 15]. In the present study, metabolic acidosis was achieved in the acidotic groups given the 1.8% ammonium chloride solution as drinking water, which was confirmed by decrease in blood pH and HCO_3^- concentration in comparison with those in control groups.

Acidotic groups in all diets showed weight loss during 5 to 10 days of beginning of experiment. This result is in agreement with data of Gafter *et al.* [11] in acidotic (1.8% ammonium chloride) rats. Metabolic acidosis suppresses the secretion of growth hormone and insulin-like growth factors [5, 6]; therefore, growth retardation in our experiment might be affected by these factors. Growth retardation in the acidotic group of LCD was more marked when compared to other groups. It might result from the severe metabolic acidosis (Table 1) by low Ca diet [1].

It is a generally accepted fact that high dietary Ca intake suppresses plasma $1,25\text{-(OH)}_2\text{D}$ level, whereas low dietary Ca intake increases it [1, 12]. On the other hand, metabolic acidosis increases plasma $1,25\text{-(OH)}_2\text{D}$ level [11] or decreases it [8]. In the present study, plasma $1,25\text{-(OH)}_2\text{D}$ level was inversely correlated with dietary Ca content but did not differ significantly in the acidotic groups from that in the control groups in all diets. These results suggest that plasma $1,25\text{-(OH)}_2\text{D}$ level may be affected by dietary Ca content, but not by metabolic acidosis. The level of plasma ionized Ca in the acidotic groups was higher compared with that in the control groups regardless of dietary Ca content in the present study. Thus, the increase in plasma ionized Ca under metabolic acidosis might be affected by humoral changes such as blood pH rather than the effect of $1,25\text{-(OH)}_2\text{D}$.

In the present study, the levels of total Ca and P in plasma were similar in the acidotic and control groups. These results agreed with data of previous researchers [1, 15]. Urinary P excretion tended to be higher in the acidotic groups than that in the control groups except for HCD, although this difference was not significant statistically. Gafter *et al.* [11] demonstrated reduced urinary P excretion in metabolic acidosis for 9 days but unchanged for 3 days. In contrast, Levine *et al.* [15] noted increased excretion in metabolic acidosis for 3 days. Data of Bushinsky *et al.* [1] showed that urinary P excretion may be affected by concentrations of ammonium chloride used to produce metabolic acidosis. Further studies will be necessary to elucidate the effects of metabolic acidosis on P metabolism.

Ca is released from bone *in vitro* when the pH of the medium is reduced [2–4, 10]; the lower the pH, the greater the Ca efflux [10]. In the present study, the increases in the urinary Ca excretion and plasma ionized Ca in the acidotic groups, therefore, suggest the increase in bone Ca resorption. The increase in Ca resorption from bone in the acidotic groups is supported by significant decrease of femur breaking force and bone volume, and significant enlargement of trabecular separation as compared with those

in the control groups in LCD, LmCD and SCD. Metabolic acidosis inhibits renal tubule Ca reabsorption [18]. The inhibition of Ca uptake in the kidneys, therefore, may result in the increase in urinary Ca excretion. On the other hand, the failure of normal osteoid mineralization may be caused by metabolic acidosis [17, 20] and vitamin D deficient and/or a low Ca diet [9]. Kraut *et al.* [14] reported that the disturbance of osteoid mineralization is not caused by a direct consequence of metabolic acidosis in thyroparathyroidectomized rats. In the present study, osteoid thickness in the acidotic groups did not significantly differ from that in the control groups in all diets. However, osteoid thickness in the acidotic groups was significantly higher in LCD than that in LmCD, HmCD and HCD. Mineral apposition rate in the acidotic groups was significantly higher in LCD and LmCD than that in HmCD and HCD. Whereas, such changes were not recognized among the control groups. Our results suggest that bone mineral loss under metabolic acidosis may be accelerated by disturbance of osteoid mineralization with high turnover of bone metabolism due to insufficient Ca intake, which may result in the decrease of mineralized bone.

Urinary Ca excretion in the acidotic groups was about 2 to 3 times higher in comparison with that in the control groups in HmCD and HCD, and plasma ionized Ca level was elevated. Mineral apposition rate and bone formation rate were lower in the acidotic groups than those in the control groups in HmCD and HCD as indicated that metabolic acidosis decreases the mineralization [14]. In spite of these facts, bone volume, trabecular separation, femur breaking force, and femur Ca and P contents in the acidotic groups in HmCD and HCD were not affected. These results suggest that bone mineral loss under metabolic acidosis can be prevented by adequate supplementation of Ca.

In conclusion, our data suggest that bone mineral loss and bone fragility under metabolic acidosis may be accelerated by failure of mineralization with high turnover of bone metabolism due to insufficient dietary Ca intake, but can be prevented by adequate supplementation of Ca to diet.

REFERENCES

1. Bushinsky, D. A., Favus, M. J., Schneider, A. B., Sen, P. K., Sherwood, L. M., and Coe, F. L. 1982. Effects of metabolic acidosis on PTH and $1,25\text{-(OH)}_2\text{D}_3$ response to low calcium diet. *Am. J. Physiol.* 243: F570–F575.
2. Bushinsky, D. A., Goldring, J. M., and Coe, F. L. 1985. Cellular contribution to pH-mediated calcium flux in neonatal mouse calvariae. *Am. J. Physiol.* 248: F785–F789.
3. Bushinsky, D. A., Krieger, N. S., Geisser, D. I., Grossman, E. B., and Coe, F. L. 1983. Effects of pH on bone calcium and proton fluxes *in vitro*. *Am. J. Physiol.* 245: F204–F209.
4. Bushinsky, D. A. and Sessler, N. E. 1992. Critical role of bicarbonate in calcium release from bone. *Am. J. Physiol.* 263: F510–F515.
5. Challa, A., Chan, W., Krieg, R. J. Jr., Thabet, M. A., Liu, F.,

- Hintz, R. L., and Chan, J. C. M. 1993. Effect of metabolic acidosis on the expression of insulin-like growth factor and growth hormone receptor. *Kidney Inter.* 44: 1224-1227.
6. Challa, A., Krieg, R. J. Jr., Thabet, M. A., Veldhuis, J. D., and Chan, J. C. M. 1993. Metabolic acidosis inhibits growth hormone secretion in rats: mechanism of growth retardation. *Am. J. Physiol.* 265: E547-E553.
7. Chen, P. S., Toribara, T. Y., and Warner, H. 1956. Microdetermination of phosphorus. *Anal. Chem.* 28: 1756-1758.
8. Ching, S. V., Fettman, M. J., Hamar, D. W., Nagode, L. A., and Smith, K. R. 1989. The effects of chronic dietary acidification using ammonium chloride on acid-base and mineral metabolism in the adult cat. *J. Nutr.* 119: 902-915.
9. Cotran, R. S., Kumar, V., and Robbins, S. 1989. Nutritional disease. pp. 438-449. In: Robbins Pathologic Basis of Disease. 4th ed. (Cotran, R. S., Kumar, V., and Robbins, S. eds.). W. B. Saunders Company, Philadelphia.
10. Dominquez, J. H. and Raisz, L. G. 1979. Effects of changing hydrogen ion, carbonic acid, and bicarbonate concentrations on bone resorption *in vitro*. *Cal. Tissue Res.* 29: 7-13.
11. Gafer, U., Kraut, J. A., Lee D. B. N., Silis, V., Walling, M. W., Kurokawa, K., Haussler, M. R., and Coburn, J. W. 1970. Effect of metabolic acidosis on intestinal absorption of calcium and phosphorus. *J. Nutr.* 239: G480-G484.
12. Green, H. B., Horst, R. L., Beitz, D. C., and Littledike, E. T. 1981. Vitamin D metabolites in plasma of cows fed a prepartum low calcium diet for prevention of parturient hypocalcemia. *J. Dairy Sci.* 64: 217-226.
13. Kraut, J. A., Mishler, D. R., and Kurokawa, K. 1984. The effect of colchicine and calcitonin on the calcemic response to metabolic acidosis. *Kidney Inter.* 25: 608-612.
14. Kraut, J. A., Mishler, D. R., Singer, F. R., and Goodman, W. G. 1986. The effects of metabolic acidosis on bone formation and bone resorption in the rat. *Kidney Inter.* 30: 694-700.
15. Levine, B. S., Ho, K., Kraut, J. A., Coburn, J. W., and Kurokawa, K. 1983. Effect of metabolic acidosis on phosphate transport by the renal brush-border membrane. *Biochim. Biophys. Acta* 727: 7-12.
16. Malluche, H. H., Sherman, D., Meyer, W., and Massary, S. G. 1982. A new semiautomatic method for quantitative static and dynamic bone histology. *Calcif. Tissue Int.* 34: 439-448.
17. Maulten, C., Montoreano, R., and Labarrere, C. 1976. Early skeletal effect of alkali therapy upon the osteomalacia of renal tubular acidosis. *J. Clin. Endocrinol. Metab.* 42: 875-881.
18. McDougal, W. S. and Koch, M. O. 1989. Effect of sulfate on calcium and magnesium homeostasis following urinary diversion. *Kidney Int.* 35: 105-115.
19. Moore, E. W. 1970. Ionized calcium in normal serum, ultrafiltrates, and whole blood determined by ion-exchange electrode. *J. Clin. Invest.* 49: 318-334.
20. Pines, K. L. and Mudge, G. H. 1951. Renal tubular acidosis with osteomalacia. *Am. J. Med.* 11: 302-311.
21. Pybus, J. 1969. Determination of calcium and magnesium in serum and urine by atomic absorption spectrophotometry. *Clin. Chem. Acta* 23: 309-317.
22. Reinhardt, T. A., Horst, R. L., Orf, J. W., and Hollis, B. W. 1984. A microassay for 1,25-dihydroxyvitamin D not requiring high performance liquid chromatography: application to clinical studies. *J. Clin. Endocrinol. Metab.* 58: 91-98.
23. Tasker, J. B. 1980. Fluids, electrolytes, and acid-base balance. pp. 415-426. In: Clinical Biochemistry of Domestic Animals, 3rd ed. (Kaneko, J. J. ed.), Academic Press, New York.
24. Yamori, Y., Fukuda, S., Tsuchikura, S., Ikeda, K., Nara, Y., and Horie, R. 1991. Stroke-prone SHR (SHRSP) as a model for osteoporosis. *Clin. and Exper. Hyper. — Theory and Practice.* A13: 755-762.