JOURNAL OF BONE AND MINERAL RESEARCH
Volume 1, Number 3, 1986
Mary Ann Liebert, Inc., Publishers

Differential Effect of Caffeine Administration on Calcium and Vitamin D Metabolism in Young and Adult Rats

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ABSTRACT

Since coffee drinking may lead to a worsening of calcium balance in humans, we studied the serial changes of serum calcium, PTH, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) and calcium balance in young and adult rats after daily administration of caffeine for 4 weeks.

In the young rats, there was an increase in urinary calcium and endogenous fecal calcium excretion after four days of caffeine administration that persisted for the duration of the experiment. Serum calcium decreased on the fourth day of caffeine administration and then returned to control levels. In contrast, the serum PTH and $1,25(OH)_2D$ remained unchanged initially, but increased after 2 weeks of caffeine administration. The intestinal absorption coefficient of calcium remained unchanged, instead of declining gradually as observed in the young control group. This finding suggests that the intestinal absorption of calcium was stimulated by the increase in $1,25(OH)_2D$ production after chronic administration of caffeine.

In the adult rat group, an increase in the urinary calcium and endogenous fecal calcium excretion and serum levels of PTH was found after caffeine administration. However, the serum $1,25(OH)_2D$ levels and intestinal absorption coefficient of calcium remained the same as in the adult control group. A decrease in the net balance of calcium occurred as a result of increased calcium excretion.

The current study, using an animal model, supports the suggestion that chronic administration of caffeine could lead to negative calcium balance when there is an impaired ability to increase the efficiency of calcium absorption. Such a situation exists in elderly human subjects, since they have a reduced capacity to synthesize $1,25(OH)_2D$.

INTRODUCTION

NEGATIVE CALCIUM BALANCE has been associated with caffeine intake in perimenopausal women.[1] A higher caffeine intake has been found in osteoporotic patients as compared to age-matched controls.[2] These observations suggest the possibility that caffeine intake may lead to a deterioration of calcium balance and may contribute to the development of osteoporosis.

Recently, our laboratory observed that urinary excretion and intestinal endogenous excretion of calcium increases in young rats after chronic administration of caffeine.[3] This result is consistent with similar findings in human studies.[1] However, the chronic administration of caffeine to young rats resulted in an increase in intestinal calcium absorption, which compensated for the urinary loss of calcium. As a result, the calcium balance of the young rats did not decrease following caffeine administration. The current study was stimulated by the possibility that the findings in the animal studies differed from those in the human studies as a result of an age-related inability in older humans to increase calcium absorption.

Calcium absorption is regulated by a negative feedback control system. The parathyroid glands serve as a calcium-sensing organ which, by secretion of parathyroid hormone (PTH), stimulates the activity of the enzyme 25-hydroxy-D-1α-hydroxylase in the kidney.[4] Renal 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) production is stimulated during

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periods of high calcium demand such as growth,[5,6] pregnancy,[7] or low-calcium intake.[4,5,8] This steroid, 1,25-$(OH)_2D$, is then rapidly localized in the intestine and enhances absorption of calcium and phosphate.

A negative calcium balance can be found in older rats fed a low-calcium diet.[9] Considerable evidence suggests that there is an age-related decline in the efficiency of intestinal calcium absorption in rats[10] and humans.[11] In addition, there is an age-related decline of renal production of 1,25-$(OH)_2D$ and intestinal calcium absorption efficiency in response to a low-calcium diet.[5,12]

We, therefore, hypothesized that caffeine administration to older rats may lead to negative calcium balance because of their inability to compensate for calcium loss. The present study was designed to test this hypothesis by comparing the sequential changes of serum calcium, PTH, 1,25-$(OH)_2D$ levels, and intestinal calcium absorption in young and adult rats during chronic administration of caffeine.

MATERIALS AND METHODS

Male Sprague–Dawley rats, either after weaning (4 weeks old) or at 12 to 13 months old, were fed a diet containing 0.5% calcium and 0.54% phosphorus (Teklad diet TD 81319). After 1 week of adaptation to the diet, the animals were divided into control and caffeine-treated groups. Caffeine was administered subcutaneously to the rats at a dose of 10 mg/$(0.1\ kg)^{3/4}$ metabolic body weight[13] daily during the experimental period. The control group received vehicle subcutaneously each day.

Calcium metabolism was studied by the method of Hurley et al.,[14] which combined a kinetic study using ^{45}Ca with a short-term classical balance study. Each study included six rats per group. They were given an intravenous injection containing 20 μCi of ^{45}Ca as $CaCl_2$. A 48-hr urine was collected for the measurement of urinary calcium, radioactivity, and creatinine. Body weight and food intake were recorded daily. Feces were collected during a 48-hr period between 6 and 54 hrs after the injection of ^{45}Ca. The feces were marked by administering 0.1 ml of 10% carmine solution by gavage at the beginning and the end of the 48-hr period. The animals were sacrificed when the balance study was completed and the serum samples were collected for analysis.

Samples of the diet and the feces were ashed in a muffle furnace at 560°C for 24 hrs, then brought up to volume with 2 N HCl. Serum, urinary, fecal, and dietary calcium were measured by atomic absorption spectrophotometry (Perkin-Elmer 560). Radioactivity was measured using a liquid scintillation counter (LKB 1210).

Calcium balance was estimated as the difference between the ingested and the urinary + total fecal calcium. The true absorbed calcium and endogenous fecal calcium were measured with the aid of ^{45}Ca.[14] The endogenous fecal Ca was determined through the relationship $Vf = Vu \times Rf/Ru$, where Vf is the endogenous fecal Ca, Vu is the urinary Ca, and Rf and Ru are, respectively, the total radioactivity of the feces and urine in the 48 hrs following the ^{45}Ca injection. True absorbed calcium was measured as the difference between ingested Ca + Vf and the total fecal calcium. The absorption coefficient of Ca (in percentage) was measured as the ratio (true absorbed Ca/Ca ingested) × 100.

Serum vitamin D metabolites were measured by the method of Shepard et al.[15] Immunoreactive PTH concentrations were measured using a double antibody heterologous radioimmunoassay.[16] The antibody against bovine PTH was derived from guinea pigs. The assay is specific toward the carboxy-terminal sequence of parathyroid hormone. Cross reaction with the aminoterminal portion of the peptide is negligible. Human PTH was used as the standard. Accordingly, this standard curve was used to determine rat serum iPTH in human PTH equivalents.[17] Student's t-test was employed for the statistical evaluation of the data.[18] Two-way analysis of variance (ANOVA) with repeated observations was used to compute the F-ratio where appropriate.[19]

RESULTS

Table 1 shows the effect of chronic administration of caffeine on body weight, food intake, and the metabolism of calcium in the young growing rats. During the 4-week experimental period, the young growing control rats had a linear gain in body weight. The total fecal Ca, endogenous fecal Ca, true Ca absorption, and net Ca balance were increased in proportion to the increase in food intake (expressed as Ca ingested).

In contrast with the control group, food intake and body weight did not increase following 4 days' administration of caffeine. The suppression of food intake and weight gain by caffeine decreased with time. Administration of caffeine resulted in an increase in the excretion of endogenous fecal Ca and urinary Ca which was apparent by day 4 and sustained during the 4-week period of the experiment. However, one week's administration of caffeine to the young growing rats had no effect on the intestinal absorption coefficient of calcium. This increase in the excretion of calcium without a change in the absorption of calcium resulted in a decrease in the net balance of calcium after the first week's administration of caffeine. Net calcium balance after one week of caffeine was significantly lower than in the control group.

There was a gradual decrease in the intestinal absorption coefficient of calcium observed in the young growing control rats of from 5 to 9 weeks of age. However, this pattern was not apparent in the caffeine-treated group. The absorption coefficient of calcium of the caffeine-treated rats became higher than the control and reached a significant level ($p < .01$) when applying the ANOVA test to the 4-week repeated measurement. The increase in the absorption coefficient of calcium after 2 weeks' administration of caffeine compensated for the increase in the excretion and resulted in no decrease in the net balance of calcium.

In contrast to the young growing rats, adult control animals experienced no significant change in body weight, food intake, and fecal and urinary calcium excretion dur-

TABLE 1. EFFECT OF CAFFEINE ADMINISTRATION ON THE ABSORPTION AND EXCRETION OF CALCIUM, URINE VOLUME, AND THE BODY WEIGHT IN THE YOUNG RATS (STARTING AT 5 WEEKS OLD)

Day	*Treatment*	*Body weight (gm)*	*Ca ingested (mg/day)*	*Total fecal Ca (mg/day)*	*Endogenous fecal Ca (mg/day)*	*True Ca abs (mg/day)*	*Urinary Ca (mg/day)*	*Abs coef (%)*	*Net balance (mg/day)*	*Urine volume (ml/day)*
0	Control	133 ± 2.4	50.1 ± 2.8	13.3 ± 1.3	2.1 ± 0.15	38.9 ± 2.0	0.55 ± 0.07	77.6 ± 1.3	36.2 ± 2.1	5.4 ± 0.82
4	Control	151 ± 2.9	60.7 ± 2.4	15.9 ± 1.1	2.4 ± 0.17	47.7 ± 2.1	0.70 ± 0.06	78.2 ± 1.6	44.4 ± 1.8	6.5 ± 0.67
	Caffeine	138 ± 3.4[b]	55.4 ± 1.7	15.3 ± 1.0	3.1 ± 0.27	43.2 ± 2.0	1.36 ± 0.13[b]	78.0 ± 1.8	38.7 ± 1.4[a]	10.4 ± 1.52[a]
7	Control	167 ± 2.5	68.4 ± 2.0	19.1 ± 1.2	2.7 ± 0.17	52.0 ± 1.9	0.64 ± 0.04	76.5 ± 1.7	49.5 ± 1.6	6.7 ± 0.79
	Caffeine	144 ± 3.4[b]	64.8 ± 1.7	18.6 ± 1.0	4.2 ± 0.33[b]	50.4 ± 1.8	1.85 ± 0.13[b]	77.8 ± 1.7	44.5 ± 1.5[a]	11.3 ± 1.34[a]
14	Control	209 ± 2.8	76.3 ± 1.7	23.4 ± 1.3	3.0 ± 0.21	55.9 ± 1.6	0.71 ± 0.04	73.3 ± 1.8	52.2 ± 1.4	7.4 ± 0.53
	Caffeine	175 ± 3.8[b]	74.4 ± 1.7	22.3 ± 1.0	4.9 ± 0.34[b]	56.9 ± 1.7	1.83 ± 0.2[b]	76.6 ± 1.6	50.0 ± 1.5	12.4 ± 2.35[a]
28	Control	293 ± 3.1	78.9 ± 2.2	28.7 ± 1.4	3.2 ± 0.15	53.4 ± 1.7	0.75 ± 0.05	67.8 ± 1.8	49.9 ± 1.7	7.2 ± 1.06
	Caffeine	264 ± 4.2[b]	77.3 ± 1.7	23.4 ± 1.3[a]	5.2 ± 0.37[b]	59.1 ± 1.9[a]	2.13 ± 0.21[b]	76.5 ± 2.1[b]	51.8 ± 1.8	14.3 ± 1.85[b]
	F-test for caffeine treatment	$p < .01$	$p < .05$	$p < .05$	$p < .01$	NS	$p < .01$	$p < .01$	$p < .05$	$p < .01$

Values are mean ± SE of six observations.
Caffeine was injected subcutaneously at a dose of 10 mg per $(0.1 \text{ kg})^{3/4}$ daily.
[a,b]Significantly different from the respective control by Student's *t*-test ($p < .05$; $p < .01$).
F-test is by two-way analysis of variance with six observations in each cell and repeated measurement at day 0, 4, 7, 14, and 28.[19]

TABLE 2. EFFECT OF CAFFEINE ADMINISTRATION ON THE ABSORPTION AND EXCRETION OF CALCIUM, URINE VOLUME, AND THE BODY WEIGHT IN THE ADULT RATS (12–13 MONTHS OLD)

Day	*Treatment*	*Body weight (gm)*	*Ca ingested (mg/day)*	*Total fecal Ca (mg/day)*	*Endogenous fecal Ca (mg/day)*	*True Ca abs (mg/day)*	*Urinary Ca (mg/day)*	*Abs coef (%)*	*Net balance (mg/day)*	*Urine volume (ml/day)*
0	Control	363 ± 4.1	90.7 ± 2.3	62.3 ± 3.2	3.97 ± 0.10	32.4 ± 1.9	1.13 ± 0.14	35.7 ± 1.4	27.3 ± 1.8	16.5 ± 2.10
4	Control	367 ± 4.3	87.4 ± 2.1	60.8 ± 2.7	4.13 ± 0.25	30.7 ± 2.0	1.20 ± 0.23	35.2 ± 1.6	25.5 ± 2.0	13.7 ± 1.24
	Caffeine	350 ± 4.7[a]	84.1 ± 2.4	59.7 ± 3.5	6.07 ± 0.36[b]	30.5 ± 2.3	1.93 ± 0.26	36.2 ± 1.7	22.6 ± 2.1	19.8 ± 2.58[a]
7	Control	365 ± 3.8	91.7 ± 1.9	64.1 ± 2.6	3.81 ± 0.27	31.4 ± 1.8	1.05 ± 0.13	34.2 ± 1.3	26.4 ± 1.7	15.4 ± 1.15
	Caffeine	358 ± 4.8	83.5 ± 2.3[a]	62.3 ± 3.3	5.84 ± 0.38[b]	27.0 ± 2.2	2.64 ± 0.21[b]	32.4 ± 1.4	18.7 ± 2.0[b]	23.4 ± 2.75[b]
14	Control	369 ± 4.3	91.3 ± 1.7	62.9 ± 3.3	4.06 ± 0.31	32.5 ± 1.9	1.21 ± 0.11	35.6 ± 1.5	27.3 ± 1.6	14.1 ± 1.76
	Caffeine	358 ± 4.6	85.7 ± 2.2	63.2 ± 3.7	6.37 ± 0.42[b]	28.9 ± 2.2	3.06 ± 0.24[b]	33.7 ± 1.7	19.5 ± 2.1[b]	26.7 ± 2.18[b]
28	Control	364 ± 3.9	89.9 ± 1.7	62.0 ± 2.8	4.15 ± 0.27	32.0 ± 1.7	1.10 ± 0.09	35.7 ± 1.3	26.9 ± 1.5	15.7 ± 1.35
	Caffeine	360 ± 4.5	85.2 ± 2.3	61.7 ± 3.5	6.01 ± 0.43[b]	30.5 ± 1.9	3.17 ± 0.26[b]	35.8 ± 1.6	20.3 ± 2.0[a]	25.2 ± 1.79[b]
	F-test for caffeine treatment	$p < .01$	$p < .01$	NS	$p < .01$	$p < .05$	$p < .01$	NS	$p < .01$	$p < .01$

Values are mean ± SE of six observations.
Caffeine was injected subcutaneously at a dose of 10 mg per $(0.1\ kg)^{3/4}$ daily.
[a;b]Significantly different from the respective control by Student's *t*-test ($p < .05$; $p < .01$).
F-test is by two-way analysis of variance with six observations in each cell and repeated measurement at day 0, 4, 7, 14, and 28.[19]

ing the 4-week experimental period. Four to seven days' administration of caffeine to adult rats resulted in a significant decrease in body weight and food intake but an increase in the endogenous fecal and urinary calcium excretion. The suppression of food intake by caffeine was sustained during the 4-week experimental period as was the increase of the excretion of endogenous fecal and urinary calcium excretion. However, the intestinal absorption coefficient of calcium was not changed during the period of caffeine administration. The net balance of calcium was lower in the caffeine-treated group as compared to the control group during the 4-week study.

Serum calcium concentration remained relatively constant in the young growing and adult control rats over the 28-day study period. In contrast, serum calcium concentration in the caffeine-treated young animals exhibited an initial decline to levels significantly lower than control values on day 4, but then returned toward control levels thereafter (Fig. 1). Serum calcium concentration in the caffeine-treated adult animals was not significantly different from the control levels over the experimental study period.

The basal levels of serum iPTH of the adult rats were higher than in the young rats. Both levels remained relatively constant over the 28-day study period without administration of caffeine (Fig. 2). Administration of caffeine resulted in an increase in serum iPTH in both adult and young rats. This increase became statistically significant by 2 weeks in the caffeine-treated young rats and by 3 weeks in the caffeine-treated adult animals.

In contrast to the serum iPTH, the serum $1,25(OH)_2D$ level in the adult rats was lower than in the young rats and both levels remained constant over the 28 days when caffeine was not administered (Fig. 3). Administration of caffeine to young growing rats resulted in no effect on the serum levels of $1,25(OH)_2D$ at the first week, followed by a significant increase two weeks later. However, an increase in serum $1,25(OH)_2D$ after caffeine administration was not observed in the adult animals.

DISCUSSION

The present experiment demonstrates that the effect of caffeine on calcium metabolism differs in young and adult rats. The excretion of endogenous fecal calcium and urinary calcium was higher and the calcium intake (food intake) was lower in both the young and adult rats that received caffeine chronically. However, caffeine increased the intestinal absorption coefficient of calcium only in the young growing group. An initial decrease in serum calcium levels was apparent in the young growing rats, corresponding to the increase in the calcium excretion and the decrease in the intake of calcium after 4 to 7 days of caffeine administration. Serum levels of PTH and $1,25(OH)_2D$ remained unchanged initially, followed by a significant increase 2 weeks after caffeine administration. Thus, it is not surprising that intestinal absorption of calcium was enhanced in caffeine-treated young rats as compared to the age-matched control group.

The dosage of the caffeine used in the current rat experiment, 10 mg/(0.1 kg)$^{3/4}$, is equivalent to 1360 mg/(70 kg)$^{3/4}$ in humans when the conversion is based on metabolic body weight.[13] If the average caffeine content in coffee is 85 mg/cup, this dosage is equivalent to the consumption of 16

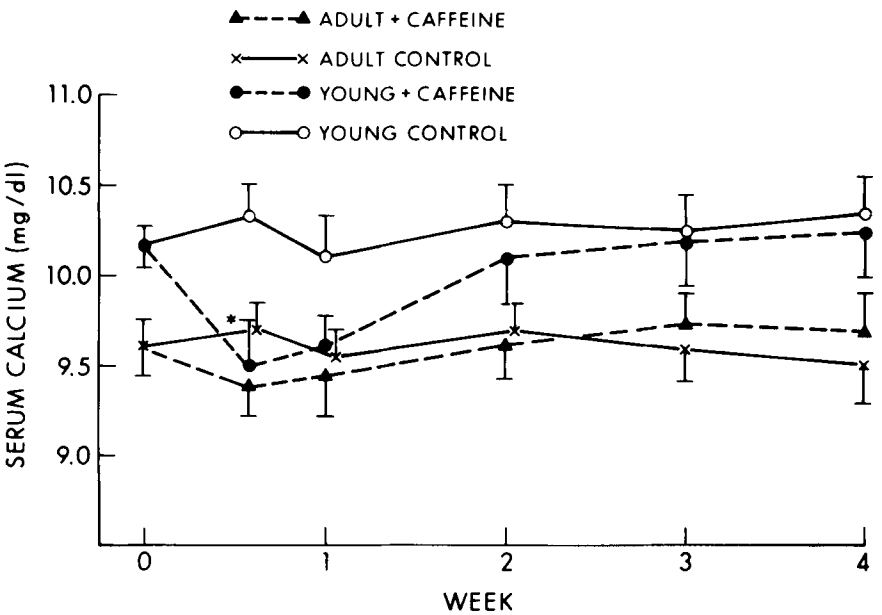

FIG. 1. Effects of caffeine administration on the serum levels of calcium in young and adult rats. Values are mean ± S.E. of six observations. The daily dosage of caffeine administered was 10 mg per (0.1 kg)$^{3/4}$ body weight. * = significantly different from the young control at day 4 by Student's *t*-test ($p < .05$).

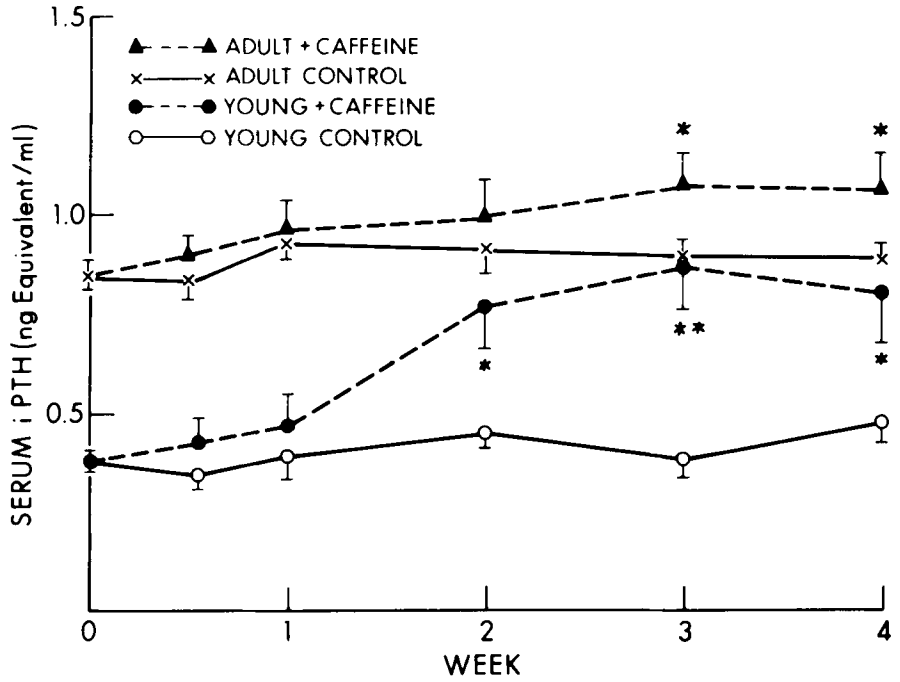

FIG. 2. Effects of caffeine administration of the serum levels of parathyroid hormone (iPTH) in young and adult rats. Values are mean ± S.E. of six observations. The daily dosage of caffeine administered was 10 mg per $(0.1\ kg)^{3/4}$ body weight. *;** = significantly different from the respective control group by Student's *t*-test ($p < .05$; $p < .01$).

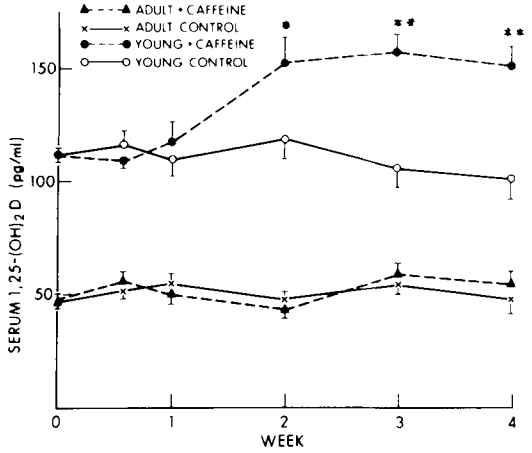

FIG. 3. Effects of caffeine administration on the serum levels of $1,25(OH)_2D$ in young and adult rats. Values are mean ± S.E. of six observations. The daily dosage of caffeine administered was 10 mg per $(0.1\ kg)^{3/4}$ body weight. *;** = significantly different from the respective control group by Student's *t*-test ($p < .05$; $p < .01$).

cups of coffee each day. This amount of caffeine is high for regular consumption in the human diet. Therefore, the relevance of these data for humans has to be tempered.

It has been reported that there is no effect on food intake after caffeine administration.[3,20] The current data show no significant difference in food intake between the caffeine-treated and the age-matched control using Student's *t*-test. However, the results of repeated observations over 4 weeks indicate a significant difference when ANOVA is applied. Caffeine administration resulted in a mild but persistent suppression of food intake.

Enhanced excretion of calcium could result in transient hypocalcemia, stimulation of PTH production, and an increase in levels of $1,25(OH)_2D_3$. On the other hand, decreased calcium intake could also result in a stimulation of production of $1,25(OH)_2D$. Both mechanisms may be responsible for the increase in the efficiency of intestinal calcium absorption.

Evidently, the increase in serum $1,25(OH)_2D$ in the current study was due to the enhancement of renal conversion of 25(OH)D to $1,25(OH)_2D$ since a concomitant increase in serum PTH was observed. This stimulation of $1,25(OH_2D$ synthesis is believed to occur because of the increased losses of calcium resulting from caffeine administration. An effect of caffeine administration on the degradation of $1,25-(OH)_2D$ in young rats has not yet been investigated and therefore cannot be excluded.

An inhibitory effect of caffeine on the conversion of 25(OH)D to $1,25(OH)_2D$ has recently been reported in isolated renal tubules from vitamin D-deficient chicks.[21] Caffeine inhibits intracellular calcium concentration which suppresses 25(OH)D-1α-hydroxylase activity in mitochondria. Nevertheless, under *in vivo* conditions we observed an increase in serum $1,25(OH)_2D$ levels in young rats after chronic administration of caffeine.

A gradual decrease in the intestinal absorption coefficient of calcium was observed in the young growing rat during the 5–9-week-old period without caffeine administration (Table 1). This result is consistent with previous evi-

dence claiming that rats over 6 weeks old show a decrease in calcium transported by the saturable component, whereas a constant proportion of the ingested calcium is transported by the nonsaturable component.[22,23]

Irrespective of the increase in calcium excretion and serum levels of iPTH and the decrease in food intake after caffeine administration, the serum $1,25(OH)_2D$ levels and intestinal absorption coefficient of calcium in the adult rats remained unchanged. intestinal calcium transport and renal production of $1,25(OH)_2D$ decrease with age.[5,10,12] We believe that the failure to observe an increase of serum $1,25-(OH)_2D$ after caffeine administration in the adult rat was due to the age-related decline in renal production of 25(OH)D-1α-hydroxylase. On the other hand, the data in Fig. 2 suggest that the response of serum PTH to the administration of caffeine was lower in the adult rats than in the young rats. Perhaps the increase in PTH in adult rats was insufficient to cause increased renal production of $1,25(OH)_2D$. Moreover, the biological activity of the circulating forms of immunoreactive PTH in young versus old rats is not known. The decrease in the net balance of calcium in the caffeine-treated adult rats is apparently due to both the age-related decline in the renal production of $1,25-(OH)_2D$ and the impaired ability to increase intestinal calcium absorption in response to the loss of calcium.

The observed increase in the excretion of calcium after caffeine administration confirms previous reports in human and animal studies.[1-3] This increase in the excretion of calcium was also apparent in both young and adult animals. In addition, adult rats showed a decline in renal production of $1,25(OH)_2D$ and in intestinal absorption of calcium. All these factors contributed to the observed decrease in the net calcium balance in the caffeine-treated adult rat.

The inability to synthesize sufficient amounts of $1,25-(OH)_2D$ to meet the needs for calcium balance is a primary event in the aging process.[5,11,24] Many older people consume diets which are deficient in calcium.[25-27] Enhancement of calcium excretion in urine and feces after the consumption by the elderly of beverages containing caffeine could accelerate the problem of negative balance of calcium. The present study in an animal model supports the suggestion that chronic administration of caffeine to the adult human could lead to a gradual deterioration of calcium balance.

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Received for publication October 7, 1985; in revised form December 16, 1985; accepted January 13, 1986.