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The Effect of High or Low Dietary Calcium on Bone and Calcium Homeostasis in Young Male Rats

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Summary: Young male rats (100 g body weight) were fed diets containing varying amounts of calcium. Body weight and bone development were studied together with various endocrine parameters, including blood levels of Ca^{2+}, calcitonin, parathyroid hormone, vitamin D, and gastrin, and the enterochromaffin-like (ECL) cell-related parameters gastric mucosal histidine decarboxylase activity and histamine concentration. A diet containing 0.5% calcium resulted in optimum body weight gain and bone development. A lower calcium intake impaired body weight gain and bone development. The impairment was manifested in reduced bone calcium content whereas the size of the bones was unaffected. The net absorption of calcium seemed to be proportional to the calcium intake. A low calcium diet (0.03%) raised the circulating levels of $1,25(OH)_2D$ and parathyroid hormone and lowered $25(OH)D_3$ and Ca^{2+}, whereas a high calcium diet (5.46%) raised calcitonin, Ca^{2+}, $25(OH)D_3$, and $1,25(OH)_2D$. In addition, the low calcium diet lowered the circulating gastrin concentration and the histidine decarboxylase activity and histamine content of the ECL cells in the gastric mucosa. A high calcium diet raised the circulating gastrin concentration, but the rise was not associated with an increase in the histidine decarboxylase activity and histamine content.

The stomach is thought to be important in the control of dietary calcium uptake and utilization. Gastric acid, for one thing, is assumed to improve the bioavailability of dietary Ca (cf [1]), and recently a hypothetical gastric hormone (tentatively named gastrocalcin) has been proposed to enhance the uptake of Ca^{2+} into bone [2–5]. Gastrocalcin is thought to originate in the so-called enterochromaffin-like (ECL) cells, the predominant endocrine cell population in the acid-producing part of the stomach [6]. The ECL cells are known to produce and store histamine in addition to an as yet unidentified peptide hormone (cf [7]).

The relationship between Ca intake, circulating concentrations of Ca, calciotropic hormones, and bone status is poorly understood. Thus, the clinical usefulness of Ca supplementation to promote bone formation and prevent bone loss is controversial (cf [8, 9]). Previous experiments have indicated that for optimum growth and calcification of bones in the rat, a diet containing 0.5–0.6% Ca is adequate [10]. Commercial rat food pellets contain between 1.2 and 1.5% Ca. Such a chronic excess of dietary Ca may affect the Ca regulating systems, reflected in the fact that excessive intake of Ca induces osteochondrosis in dogs [11]. A low Ca intake, on the other hand, impairs bone mineralization [10–12]. Recently, it was reported that a diet containing 0.1% Ca is enough to sustain normal growth but not bone mineralization in young female rats [12]. The purpose of the present study was to examine the consequences of low and high Ca diets on bone, Ca metabolism, and calciotropic hormones in the young male rat.

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Material and Methods

Experimental Protocol

Ninety-two male Sprague-Dawley rats, weighing about 100 g each, were divided into six groups and given rat food pellets containing 0.03% Ca (n = 27), 0.18% Ca (n = 5), 0.32% Ca (n = 5), 0.5% Ca (n = 10), 1.5% Ca (n = 30), and 5.46% Ca (n = 15), respectively. Calcium is in the form of carbonates and phosphates. Each diet has 0.7% phosphorus. Each rat received 16 g food/day (free access to tap water (62 mg calcium/liter) for 4 weeks. Blood samples for determination of Ca^{2+} and calcitonin were collected from the tip of the tail the day before the rats were sacrificed. The rats were exsanguinated via the abdominal aorta under ketamine/xylazin (Ketalar/Rompun, Parke-Davis, UK and Bayer, FRG, respectively) anesthesia, and serum was taken for analysis of parathyroid hormone (PTH), $25(OH)D_3$, $1,25(OH)_2D$, and gastrin. The stomach was dissected out, rinsed with 0.9% saline, and the oxyntic mucosa was scraped off the gastric wall with a scalpel, frozen on dry ice, and weighed. This material was used for determination of histamine and histidine decarboxylase activity. Radius, tibia, and femur were dissected out, cleaned, and weighed. The volume was measured (femur only) according to the principle of Archimedes (using a balance and a beaker filled with redistilled water) and the bones were placed in an oven at 800°C for 24 hours. The resulting ash was weighed and the bone density was calculated by dividing the ash weight (mg) with the volume (mm^3).

Urinary and Fecal Extraction of Calcium

Nine rats from each of the 0.03% Ca, 1.5% Ca, and 5.46% Ca groups were placed in metabolic cages for 3 days beginning 2 weeks after start of ingesting the diets. The amount of food and water ingested was measured. The urine and feces from each individual rat was collected for each 24-hour period. The urine volume and feces weight were determined and the daily excretion of Ca in urine and feces was measured. For determination of fecal calcium, feces was homogenized (Polytron) in 1 M HCl (1 g feces/10 ml acid) and left standing for 3 hours at 4°C followed by centrifugation at 15,000 × *g* for 1 hour at 4°C. The clear supernatant was diluted 1:50 with redistilled water. The Ca concentrations in urine and fecal extracts were measured by the o-cresolphthalein method (Calcium C, Wako

Chemicals, Neuss, FRG) and the daily calcium excretion for each rat was calculated. The net absorption of Ca was calculated from the difference between intake and excretion. The mean values (intake, excretion, and net absorption) for each individual were calculated by averaging the results of the daily determinations during the 3-day period. Group means were calculated from the individual means.

Blood Sampling

For collection of blood, the rats were placed in Bollman-type restraining cages and injected s.c. in the proximal part of the tail with a local anesthetic, bupivacaine (Marcaine, Astra, Sweden), 1 hour before sampling. Blood (150–300 μl) was drawn from the tip of the tail.

Blood Ca^{2+} Determination

Ca^{2+} was determined by a Ca-selective electrode (ICA 2, Radiometer, Copenhagen, Denmark) in 150-μl samples of fresh whole blood collected in heparinized capillary tubes.

Gastrin and Calcitonin Radioimmunoassays (RIA)

The serum gastrin concentration and the serum calcitonin concentration were determined as described elsewhere [13–15].

PTH RIA

^{0}Tyr-PTH 44-68 (human) was radioiodinated by chloramine-T oxidation. The resulting tracer was purified by HPLC: Waters system, μBondapak C_{18}-column, isocratic elution with 15% (vol/vol) acetonitrile in sodium phosphate buffer, pH 3.5. We used a chicken anti-PTH-mid molecule serum (Incstar, Stillwater, MN, USA.), which recognizes peptides containing the midportion of PTH. Rat serum or a standard solution containing human ^{0}Tyr-PTH 44-68 in various concentrations (16–1000 pmol/liter) was incubated with the antiserum at 4°C for 16 hours. Tracer (about 10,000 cpm) was added and the incubation was continued for another 24 hours. Antibody-bound tracer was separated from free tracer by goat anti-chicken IgG (Milab, Malmö, Sweden). The RIA has a detection limit of 20 pmol/liter. The standard curve is virtually parallel with the dilution curve of rat parathyroid extracts. Inter- and intraassay variations were 14% and 12%, respectively. The PTH concentrations were expressed as pmol equivalents of human PTH 44-68.

Determination of 25(OH)D_3 and 1,25$(OH)_2$D

The serum 25(OH)D_3 concentration was determined by an HPLC method described in detail elsewhere [16, 17]. 1,25$(OH)_2D_2$ and 1,25$(OH)_2D_3$ in serum were measured by a commercially available radioreceptor assay method (Incstar, Stillwater, MN, USA) after passage through a Sep-Pak C_{18} cartridge [18].

Determination of Histidine Decarboxylase Activity

The oxyntic mucosa was homogenized in ice-cold 0.1 M sodium phosphate buffer, pH 7.0, to a concentration of 100 mg wet weight/ml. 0.5 ml of each homogenate was incubated with 4×10^{-4} M (1 – ^{14}C)L-histidine (New England Nuclear) in the presence of 10^{-5} M pyridoxal-5-phosphate and 5×10^{-4} M reduced glutathione at 37°C for 1 hour under nitrogen. Total reaction volume was 0.53 ml. The amount of $^{14}CO_2$ formed during the reaction was measured by liquid scintillation counting (for details see [19]).

Determination of Histamine Concentration

The oxyntic mucosal homogenate was diluted 1:10 in 3% trichuloroacetic acid and heated in boiling water for 5 minutes to extract histamine. Precipitated material was spun down and the supernatant was diluted 1:50 with redistilled water. Aliquots of 100μl were taken for assay of histamine as described by Rönnberg and Håkanson [20].

Results

Effect of Dietary Calcium on Body Weight and Bone Development

Diets containing 0.03–0.32% calcium produced a reduced body weight ($P < 0.05$) (Fig. 1A) and bone weight and density (Fig. 1B–D), compared with diets containing 0.5 or 1.5% Ca. The volume of the femur was not much affected by the various diets (six rats in each group): 453 μl ± 17 with a diet of 0.03% Ca, 466 ± 12 with 0.5% Ca, 495 ± 19 with 1.5% Ca, and 446 ± 19 with 5.46% Ca. The rats consumed all the food they were given (16 g/day/rat) except those offered the 5.46% Ca diet; they consumed 13 g ± 1/day.

Effect of Dietary Ca on Net Absorption of Ca

The mean daily net absorption of Ca was 60% of the ingested Ca in rats fed a low Ca diet (0.03%), 45% in rats fed a standard Ca diet (1.5%), and 63% in rats fed a high Ca diet (5.46%) (Table 1).

Effect of Dietary Ca on Blood Ca, Vitamin D, and Calciotropic Hormones

The low Ca diet (0.03%) reduced the blood levels of Ca^{2+} and 25(OH)D_3, and increased the levels of 1,25$(OH)_2$D and PTH, while leaving the concentrations of calcitonin unaffected (Table 2). The high calcium diet (5.46%) raised the blood levels of Ca^{2+}, calcitonin, 25(OH)D_3, and 1,25-$(OH)_2$D, while leaving PTH unaffected (Table 2).

Effect of Dietary Ca on Gastrin and ECL Cell-Related Parameters

The low Ca diet (0.03%) reduced the serum gastrin concentration and the gastric mucosal histidine decarboxylase activity and histamine concentration. The high Ca diet (5.46%) raised the circulating levels of gastrin but not the gastric mucosal histidine decarboxylase activity (Table 3).

Discussion

Calcium homeostasis involves the interaction of at least three hormones: PTH, vitamin D, and calcitonin. High Ca concentrations in the blood are known to suppress PTH secretion whereas low Ca concentrations stimulate it [12, 20–21]. Food rich in Ca stimulates calcitonin and gastrin release [22, 23]. Recently, the histamine-producing ECL cells of the acid-producing part of the stomach have been suggested to contain a calciotropic agent, tentatively referred to as gastrocalcin, which is released in response to gastrin [2–5]. In the present study, we were interested in defining the dietary Ca requirements for normal body weight and bone development

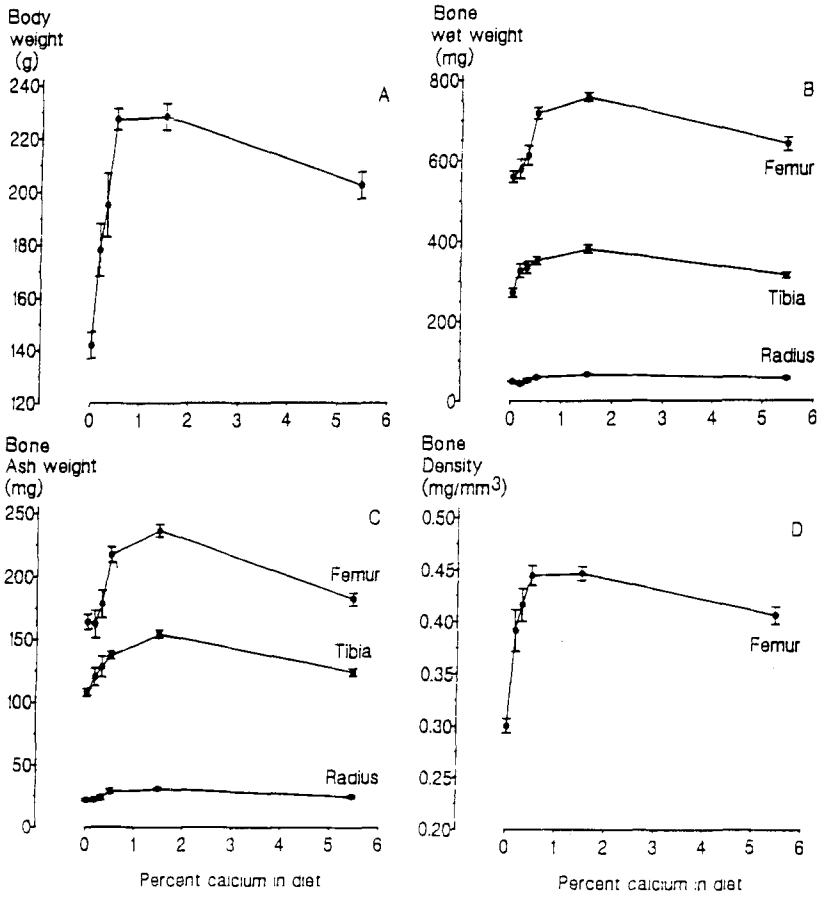

Fig. 1. Effects of diets with different Ca content on **(A)** body weight, **(B)** bone wet weight, **(C)** bone ash weight, and **(D)** bone density. Means ± SEM. Vertical bars give SEM. Each value is the mean of 5–15 determinations. Each rat received and ingested 16 g food/day for 4 weeks except those on 5.46% Ca who ingested 13 g food/day. Note that a dietary Ca content between 0.5 and 1.5% appears to be optimal for body growth and bone development.

Table 1. Effects of diets with different Ca content on the daily intake, excretion, and net absorption of Ca

Diet (% Ca)	No. of rats	Daily food intake (g)	Ca intake (mg/rat/day)	Ca excretion (mg/rat/day)	Net Ca absorption (mg/rat/day)
0.03	9	16	6 ± 0.5	3 ± 1	3 ± 1.5
1.5	9	16	266 ± 3	139 ± 25	127 ± 23
5.46	9	13 ± 1	520 ± 25	205 ± 26	315 ± 31

Means ± SEM. The rats were on the diets for 2 weeks before being placed in metabolic cages for 3 days. Each rat was given 16 g food/day. The various parameters were measured each day during those 3 days. For details see Material and Methods

in young male rats. We also wanted to examine the effects of high and low Ca diets on various endocrine parameters.

A daily intake of 16 g of a 0.5% Ca diet (total daily intake of about 450 mg Ca/kg B.W.) for a period of 4 weeks is enough to sustain normal body weight and bone development in young male rats. The standard commercial rat diet contains 1.2–1.5% Ca. A low Ca intake resulted in an impaired body weight gain and bone development. It should be noted that the impairment was in bone Ca content and not in bone size. A high Ca diet reduced food intake which may explain the slightly impaired body weight gain and bone development in these rats. The amount of Ca in the diet may have important consequences for the formation and release of various hormones in the body, notably those concerned with Ca metabolism. A high Ca diet raised the blood Ca level and the concentration of calcitonin in serum. A low-Ca diet lowered the blood Ca level; the concentration of calcitonin was unaffected. The effects on serum PTH were the reverse. The $25(OH)D_3$ concentration in serum increased with increasing blood Ca levels, as did the gastrin concentration. In view of the fact that the ECL cells in the oxyntic mucosa are under control of gastrin [24, 25], it was not unexpected that the low serum gastrin concentration in the rat given low Ca diet was associated with a low ECL cell activity, reflected in a low histidine decarboxylase activity and histamine concentration. Interestingly, however, the hypercalcemia induced by high Ca intake was not associated with high histamine content or histidine decarboxylase activity, despite the high serum gastrin concentration. It appears that the ability of the ECL cells to respond to gastrin is impaired by hypercalcemia.

Calcium absorption from the small intestines involves first, a saturable active uptake process in the duodenum regulated by $1,25(OH)_2D$ via the carrier calcium-binding pro-

Table 2. Effects of diets with different Ca content on circulating levels of Ca^{2+}, calcitonin, PTH, $25(OH)D_3$, and $1,25(OH)_2D$

Diet (% Ca)	Ca^{2+} (mmol/liter)	Calcitonin (pmol/liter)	PTH (pmol/liter)	$25(OH)D_3$ (μg/liter)	$1,25(OH)_2D$ (ng/liter)
		NS			
0.03	1.23 ± 0.01[c] (16)	12 ± 1 (25)	56 ± 3[b] (18)	9 ± 1[c] (18)	158 ± 23[a] (4)
1.5	1.32 ± 0.01 (11)	13 ± 1 (30)	40 ± 4 (11)	26 ± 1 (10)	95 ± 8 (4)
			NS		
5.46	1.39 ± 0.01[c] (5)	18 ± 1[b] (12)	34 ± 2 (7)	30 ± 1[a] (8)	130 ± 8[a] (4)

Means ± SEM. Number of rats in parenthesis. The rats were on the diets for 4 weeks and the various parameters were measured at the time of sacrifice. The statistical significance of the differences between the results obtained with the high Ca diet and the 1.5% Ca diet and between the low Ca diet and the 1.5% Ca diet are indicated. [a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.001$, NS, not significant; Student's *t*-test

Table 3. Effects of diets with different Ca content on serum gastrin concentration and gastric mucosal histidine decarboxylase (HDC) activity and histamine concentration

Diet (% Ca)	Gastrin (pmol/liter)	HDC activity (nmol $^{14}CO_2$/g w.w.)	Histamine (μg/g w.w.)
0.03	11 ± 1[c] (15)	24 ± 3[c] (27)	46 ± 2[c] (27)
1.5	50 ± 4 (18)	78 ± 9 (27)	78 ± 3 (27)
		NS	
5.46	83 ± 6[c] (9)	66 ± 9 (15)	65 ± 4[a] (15)

Means ± SEM. Number of rats in parenthesis. The rats were on the diets for 4 weeks, and the various parameters were measured at the time of sacrifice. The statistical significance of the differences between the results obtained with the high Ca diet and the 1.5% Ca diet and between the low Ca diet and 1.5% Ca diet are indicated
[a] $P < 0.05$; [c]$P < 0.001$, NS, not significant; Student's *t*-test

tein, and secondly a nonsaturable passive diffusion in the lower small intestine (cf [26]). Pansu et al. [27] demonstrated in rats that the saturable process predominated with low-to-moderate amounts of Ca in the diet, whereas the amount of Ca absorbed by the nonsaturable process increased in direct proportion to the amount of Ca ingested. In view of the elevated levels of $1,25(OH)_2D$ in the circulation of rats fed a diet low in Ca (see [11]), it appears that the saturable absorption process does indeed represent the predominant pathway under these circumstances, and that the nonsaturable absorption might be predominant in rats fed a diet high in Ca. The results obtained in the present study suggest that on the whole the net absorption of Ca is proportional to the Ca intake.

From the results, it seems that a 0.5% Ca diet supplies the minimum amount of Ca required for a normal body weight gain and bone development in young male rats. It also seems that the mechanisms that control intestinal Ca absorption do not protect against excess Ca in the diet. A low Ca diet raised the circulating levels of $1,25(OH)_2D$ and PTH and lowered Ca^{2+} and $25(OH)D_3$ whereas a high Ca diet raised Ca^{2+}, calcitonin, $1,25(OH)_2$, and $25(OH)D_3$. Also the serum gastrin concentration and the activity of the ECL cells, reflected in the histidine decarboxylase activity, were affected by the amount of Ca in the diet. A low Ca diet suppressed the serum gastrin concentration and predictably also the histidine decarboxylase activity of the ECL cells. A high Ca diet raised the serum gastrin concentration but did not produce the expected activation of the enzyme in the ECL cells.

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