PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 172, 324–329 (1983)

Dietary Protein Level and Skeletal Development in the Golden Syrian Hamster[1,2] (41564)

LINDSAY H. ALLEN, RICHARD J. WOOD, AND RICHARD S. BARTLETT

Department of Nutritional Sciences, University of Connecticut, Storrs, Connecticut 06268

Abstract. The hamster was used as a model for investigating the effect of low, moderate, and high protein intake (12, 18, and 36% casein) on bone mineral content. Animals fed the low level of protein between 3 and 8 months of age had a reduction in the weight of all skeletal components measured, with the exception of the diaphyseal portion of the long bones. Diaphyseal weight and calcium remained significantly lower when expressed as a percentage of body weight. However, urinary calcium excretion was not lower than that of animals consuming an adequate protein intake. Ingesting a high protein diet resulted in a significant increase in urinary calcium excretion, and a reduced amount of both mineral and organic material in the diaphyses. We conclude that long-term consumption of a high protein ration led to the development of a mild osteoporotic condition in the hamster which was limited to the diaphyseal portions of the long bones.

Consumption of protein causes an increased excretion of urinary calcium in both humans (1, 2) and rats (3, 4). In a long-term metabolic study, we have produced a negative calcium balance of −137 mg/day in human subjects consuming 225 g protein and 1400 mg calcium/day (5), which persisted for the 48 days during which the high protein diet was fed. The source of this calcium loss was assumed to be skeletal since intestinal absorption of calcium was not increased. However, given the methodological problems of measuring small changes in skeletal calcium *in vivo*, it was necessary to develop an appropriate animal model to evaluate the long-term effects of high protein intake on skeletal calcium homeostasis.

We have shown recently in humans that the increase in urinary calcium after protein consumption is due to a reduction in the percentage of filtered calcium reabsorbed by the kidney (6). Although the rat has been used by some investigators to study protein-induced calciuria (3, 4) we have questioned the suitability of this species since the rat excretes a very low percentage (<1%) of calcium intake via the urinary route (3) unless the dietary calcium to phosphorus ratio is high. Therefore, the calcium loss induced by protein consumption represents a relatively small loss to the rat, and a significant loss of bone calcium is unlikely to occur.

In a preliminary search for a more appropriate animal model, we found that the urinary calcium excretion of the golden Syrian hamster (*Mesocricetus auratus*) is equivalent to 20 to 30% of its calcium consumption. Since this is a similar percentage to that excreted by humans, the golden Syrian hamster was utilized to evaluate the long-term effects of different dietary protein levels on various skeletal parameters. The golden hamster ceases growth at approximately 160 days of age (8); although little is known about calcium metabolism in this species, there is evidence that intestinal absorption is related to intestinal calcium-binding activity (9), and that parathyroid hormone has a shorter biological half-life than in the rat (10). Bone determination has been reported in aged hamsters (11).

Materials and Methods. Thirty 3-month-old male Golden Syrian hamsters were randomly assigned to individual wire-bottom cages for 21 weeks. For the first 5 days, all hamsters were fed *ad libitum* a 1:1 mixture of ground rat pellets and semipurified ration (Table I) containing 18% casein. On Days 6 and 7, they were fed the semipurified ration only. This represented a 1-week standardization period. On Day 8, the beginning of the comparison period, the animals were randomly assigned to one of three rations containing either 12, 18, or 36% casein (Table I),

[1] Supported in part by U.S. Public Health Service Research Grant DE004295.

[2] Scientific Contribution No. 951, Agricultural Experiment Station, University of Connecticut, Storrs, CT 06268.

0037-9727/83 $1.50

TABLE I. COMPOSITION OF THE DIETS

Ingredient (%)	Dietary protein level		
	Low	Adequate	High
Casein[a]	12.0	18.0	36.0
Dextrin[b]	67.5	61.5	43.5
Cottonseed oil	10.0	10.0	10.0
Cellulose	4.0	4.0	4.0
Mineral mix[c]	3.5[d]	3.5[e]	3.5[f]
Vitamin mix[g]	2.0	2.0	2.0
Choline mix[h]	1.0	1.0	1.0

[a] Casein, vitamin free, General Biochemicals, Chagrin Falls, Ohio.

[b] Dextrin, General Biochemicals, Chagrin Falls, Ohio.

[c] For all diets, contains (g/kg) 7.3000 KCl; 2.3000 $MgSO_4$; 0.1540 $MnSO_4 \cdot H_2O$; 0.1300 $CuSO_4$; 0.1576 $FeC_6H_5O_7 \cdot H_2O$; 0.0010 KIO_3.

[d] Contains (g/kg) 10.2467 $CaCO_3$; 7.1213 $CaHPO_4$; 6.5100 Na_2HPO_4; 1.1819 cerelose.

[e] Contains (g/kg) 11.7979 $CaCO_3$; 5.0123 $CaHPO_4$; 6.5100 Na_2HPO_4; 0.0121 $ZnCO_3$; 1.7421 cerelose.

[f] Contains (g/kg) 15.4845 $CaCO_3$; 5.1376 Na_2HPO_4; 0.0049 $ZnCO_3$; 1.5668 $C_2H_2NaO_2$; 2.8807 cerelose.

[g] Contains per kg diet: 30.90 mg vitamin A acetate (325,000 IU/g); 2.50 mg vitamin D_3 (200,000 IU/g); 25.0 mg α-tocopheryl acetate; 6.0 mg thiamin HCl; 6.0 mg riboflavin; 6.0 mg pyridoxine HCl; 10.0 mg Ca-pantothenate; 10.0 mg nicotinic acid; 5.0 mg menadione (vitamin K_3); 0.2 mg biotin; 10.0 mg vitamin B_{12}; 2.0 mg *p*-aminobenzoic acid; 2.5 mg inositol; 19.882 g cerelose.

[h] Contains 4 g choline chloride and 6 g cerelose/kg ration.

representing a low, moderate, or high level of protein for the hamster (12). The dextrin content of the rations was adjusted to equalize the caloric value of all three diets, the nutrient content of which was adequate to meet the dietary requirements of the hamster (12). The diets, including mineral mix, contained 0.62% calcium and 0.4% phosphorus by analysis.

Food and water were available *ad libitum*. Ambient temperature was maintained at approximately 21°C and light exposure was provided at an intensity of 220 ± 5 lux for a 12-hr period (0800–2000 hr) daily. Daily food intakes were recorded, and individual body weights were obtained weekly.

Animals were placed in stainless-steel metabolic cages where urine was collected daily for two separate 4-day periods, one during week 19 and one during week 20 of the comparison period. Each 24-hr urine sample was acidified with concentrated HCl and analyzed for calcium by atomic absorption spectrophotometry.

The animals were sacrificed 20 weeks after the initiation of comparison period. Kidneys were weighed, and the remainder of the carcasses eviscerated, and autoclaved for 12 min at 121°C. The skeletons were then stripped clean of adhering tissue and defatted in a mixture of chloroform and ether (9:1) for 48 hr and dried overnight at 40°C. The weights of the total skeleton and of the following individual skeletal components were determined: skull, 12 long bones, vertebrae, 7 scapulae, pelvis, and right mandible. The long bones were subsequently separated into epiphyseal and diaphyseal portions (13).

The diaphyseal portions of the 12 long bones for each animal were dry ashed in a muffle furnace at 600°C overnight, digested with concentrated HCl, and diluted with distilled water and 5% $LaCl_3$. Calcium concentration was determined by atomic absorption spectrophotometry.

Data were analyzed by one-way analysis of variance. The least-significant difference test was used to detect differences between the low or high protein groups and the moderate protein (control) group if a significant effect of diet ($P < 0.05$) was found using the F test (14).

Results. Table II shows the effect of the dietary protein level on average weight gain and food intake. Although there was a tendency for the animals on the low protein ration to gain less weight and have higher food intakes than both the moderate and high protein groups, these differences were not found to be statistically significant.

Table III shows the average kidney weights

TABLE II. INFLUENCE OF PROTEIN LEVEL ON WEIGHT GAIN AND FOOD INTAKE

Dietary protein level	Initial body weight (g)	Terminal body weight (g)	Weight gain (g)	Food intake (g/day)
Low	110.6	148.8	38.2	8.2
Moderate	111.1	159.5	48.4	7.7
High	107.9	160.8	52.9	7.3
SEM[a]	1.9	4.0	3.9	0.3
CV[b] (%)	5.0	7.7	25.2	11.9

[a] Standard error of mean = $\sqrt{\text{error mean square}/n}$, where n = number of observations per treatment.

[b] Coefficient of variation = $\sqrt{\text{error mean square}}/\bar{x} \times 100$, where $\bar{x}$ = overall mean.

TABLE III. INFLUENCE OF PROTEIN LEVEL ON KIDNEY WEIGHT AND URINE CALCIUM

Dietary protein level	Kidney weight (g)	Kidney weight (g/100 g body wt)	Urine calcium (mg/day)	Urine calcium (mg/g kidney tissue)	Urine calcium (mg/100 g body wt/day)
Low	1.20	0.81	9.39	7.48	6.20
Moderate	1.35	0.85	9.49	7.10	6.00
High	1.48	0.92	14.50[a]	9.43[b]	9.50[a]
SEM	0.12	0.03	1.11	0.84	0.45
CV	9.0	10.1	30.1	27.6	29.7

[a] Significantly different from moderate protein group, $P < 0.001$.
[b] Significantly different from moderate protein group, $P < 0.08$.

at the time of sacrifice for the various treatment groups. There was a tendency for the weight of the kidneys to increase with increasing level of protein in the ration. However, neither the low nor the high protein group kidney weights were significantly different from those of the moderate protein group. No significant differences were found between these groups when the data were expressed on a body weight basis.

Urinary calcium excretion (Table III) was significantly greater in animals receiving the high protein ration than in those receiving the moderate or the low protein ration. No significant difference was found in urinary calcium excretion between the moderate and low protein levels. When urinary calcium excretion was expressed per gram of kidney tissue the high protein group was 33% greater than the moderate protein group. This difference was significant at the $P < 0.09$ level. The effect of the high protein ration on urinary calcium excretion was also found to be significant ($P < 0.001$) when the data were expressed on a body weight basis.

Table IV shows the effect of the different protein regimens on the weight of selected individual skeletal components, and Table V shows their effect on diaphyseal ash and calcium content. Consumption of the high protein ration caused no significant change in the weight of the total skeleton, epiphyses, ver-

TABLE IV. INFLUENCE OF DIETARY PROTEIN LEVEL ON BONE WEIGHTS (g)[a]

	Dietary protein level				
	Low	Moderate	High	SEM	CV (%)
Long bones[b]	1.4912	1.6359	1.5546[i]	0.0274	5.6
Diaphysis	0.8928[g]	1.0460	0.9253	0.0270	9.0
Epiphysis	0.5880	0.5670	0.6275	0.0170	9.0
Vertebrae[c]	1.0507[i]	1.1546	1.1592	0.0290	8.2
Mandible[d]	0.2451	0.2692	0.2735	0.0055	6.6
Scapulae	0.1722[h]	0.1981	0.1874	0.0045	7.6
Skull[e]	1.1095[i]	1.2095	1.2361	0.0038	9.1
Pelvis	0.3504[h]	0.3862	0.3705	0.0078	6.6
"Total skeleton"[f]	4.4191[g]	4.8888	4.7812	0.0073	5.2

[a] Mean values for fat-free, dry weight. Ten animals per treatment.
[b] Long bones = sum of 12 bones (2 femurs, 2 tibiae, 2 fibulae, 2 ulnae, 2 radii, 2 humeri).
[c] Vertebrae = 7 cervical, 13 thoracic, 6 lumbar, and 4 sacral. Caudal vertebrae were not included in the analysis.
[d] Left mandible only.
[e] Skull, excluding both mandibles.
[f] "Total" skeleton = sum of long bones, vertebrae, left mandible, scapulae, skull, and pelvis.
[g] Significantly different from moderate protein group, $P < 0.001$.
[h] Significantly different from moderate protein group, $P < 0.01$.
[i] Significantly different from moderate protein group, $P < 0.05$.

TABLE V. INFLUENCE OF DIETARY PROTEIN LEVEL ON DIAPHYSEAL ASH AND CALCIUM CONTENT[a]

	Dietary protein level				
	Low	Moderate	High	SEM	CV (%)
Diaphyseal ash (mg)	0.6030[b]	0.7001	0.6361[b]	0.0165	8.5
Diaphyseal ash (%)	69.0	67.5	67.9	0.6	2.5
Diaphyseal calcium (mg)	0.2015[b]	0.2474	0.2226[c]	0.0105	14.1
Diaphyseal calcium (%)	23.1	23.5	23.8	1.0	12.8

[a] Diaphyses from all 12 long bones (see Table IV, footnote *b*).
[b] Significantly different from moderate protein group, $P < 0.01$.
[c] Significantly different from moderate protein group, $P < 0.05$.

tebrae, mandible, scapulae, skull and pelvis, but did cause a significant reduction in the weight of the long bones, diaphyseal bone, diaphyseal ash, and diaphyseal calcium.

The low protein ration caused a significant reduction in the weight of all skeletal parameters investigated except epiphyseal weight and percentage diaphyseal ash and calcium. However, only diaphyseal weight, ash, and calcium were significantly lower ($P < 0.05$) in the low protein group when data were expressed as a percentage of body weight.

Discussion. The results of this study have shown that the hamster is a useful model for the study of protein-induced hypercalciuria. The daily urinary calcium excretion of animals fed the high protein ration represented 30% of their calcium intake, whereas that of animals with the moderate protein diet was equivalent to 20% of their intake. Thus, urinary levels more closely approximated the 15 to 20% of calcium intake that is excreted via the urinary route in humans. Urinary calcium was 53 and 54% higher in animals consuming the high protein ration compared to those consuming the moderate and low protein ration, respectively. The magnitude of the protein-induced calciuria in hamsters is less dramatic on a percentage basis than the 230% increase seen in rats fed a 36% compared to an 18% diet (3). However, this change is of much greater quantitative significance in the hamster because of its higher urinary calcium output. While the 200 g rat fed a 36% casein diet excretes 1.7 mg urinary calcium/day (3), our hamster excreted 14.5 mg/day at the same level of protein intake.

The low protein diet used for adult animals in this study contained 12% casein. The protein requirement of the growing hamster is between 16 and 20% of the diet (15), whereas that of the adult is closer to 6% (16). The level in our low protein diet was chosen to prevent the potential consequences of severe protein deficiency on calcium absorption and calcium-binding protein synthesis (17, 18). In addition, the food intake, and therefore calcium intake, was similar in the three groups. This is in contrast to the situation in most experiments in which the effect of a severe protein deficiency on bone composition has been investigated (17). Using this approach, we had expected that the calciuretic response of hamsters would be directly proportional to the level of dietary protein, in a dose response manner similar to that seen in humans (1). However, we found no difference between the urinary calcium excretion of hamsters fed the 12 and 18% casein diets. Although the reason for this is unknown, Bell *et al.* found a similar pattern in the rat, where urinary calcium was slightly increased when the protein intake was raised from 10 to 20%, compared to a much larger increase when it was raised to 40% of the diet (4).

The terminal body weight of animals in the low protein group was slightly (although not significantly) less than that of those receiving the adequate and high protein diets. It would therefore appear that this level of protein might not be adequate for maximum growth. The weights of all skeletal components measured, with the exception of the epiphyses, were significantly reduced in the low protein group. Diaphyseal weight and calcium content were also significantly lower when these data were expressed on a body weight basis. This is in contrast to the consequences of a severe (4% casein) protein deficiency in the growing rat, in which epiphyseal regions of bone showed

a greater reduction in weight and ash content than did diaphyseal regions (17). It is conceivable that the epiphysis of the rapidly growing animal is more severely affected than the diaphysis by protein deprivation, and in the adult, the diaphysis is more severely affected.

Consumption of the high-protein ration also resulted in a significantly lower content of ash and calcium in diaphyseal bone. However, no other bones analyzed were significantly affected by the high protein intake. The reduction in the weight of the long bones could be totally attributed to the reduction in diaphyseal bone. Since the percentages of both calcium and ash in diaphyseal bone were unaffected, the reduction in diaphyseal weight must have been due to a lower content of both mineral and organic matrix. This is the situation which occurs in osteoporosis (19). It is common for investigators to restrict their measurements of bone composition to the long bones since these are the most accessible. The fact that bone mass was lower only in the diaphyseal portion of long bones suggests that it is not always possible to generalize from data obtained from the long bones to the rest of the skeleton.

Beecher and Coupain evaluated the effects on bone mineral and breaking strength of feeding a high protein diet to rats for 6 weeks (20). In animals sacrificed at 19 weeks of age, consumption of a 45% casein diet compared to one containing 25% casein resulted in decreases in femur length, maximum breaking strength, dry weight, calcium, and phosphorus. In contrast, Engstrom and DeLuca found an increase in femur ash when 30-day-old rats were fed a 36% compared to an 18% casein diet (21).

Bell *et al.* compared the effects of feeding a high protein (40% casein) compared to an adequate protein (20% casein) diet on bone resorption in the rat (4). Bone resorption was measured indirectly by following the loss of ^{45}Ca from adult rats "deep-labeled" with repeated doses of ^{45}Ca for 1 month, prior to the feeding of the different protein levels for 98 days. No protein-induced bone resorption occurred. The investigators concluded that consumption of the high protein diet caused a shift in calcium excretion from the intestine (endogenous fecal calcium) to the urine, and an increased absorption of dietary calcium. This interpretation does not agree with our kinetic data from rats, showing that absorption and endogenous secretion of calcium were unaffected by protein level (3). In addition, high protein intakes had no effect on calcium absorption in humans (5), but caused a negative calcium balance and, presumably, a reduction in bone calcium accumulation.

Weiss *et al.* (22) recently used a different technique to demonstrate that high protein diets impaired bone formation, but had no effect on remodeling. Demineralized bone matrix prepared from diaphyses was implanted subcutaneously into 35-day-old rats fed 20 or 80% protein diets. The high-protein group had reduced bone formation, measured by ^{45}Ca accumulation, and a failure of osteoid formation and subsequent mineralization. Mesenchymal cell formation and chondrogenesis were unaffected. Therefore, it would appear as if the effect of a high-protein intake is to reduce the availability of calcium at the site of mineralization. Whether or not this is a direct effect of a higher urinary calcium excretion, or an indirect hormonal effect, remains to be determined. The detrimental consequences of an impairment in osteogenesis might be more severe in rapidly growing animals, thereby explaining the mild bone demineralization which occurred in our relatively mature hamsters.

1. Margen S, Chu U-Y, Kaufmann NA, Calloway DH. The calciuretic effect of dietary protein. Amer J Clin Nutr **27**:584–589, 1974.
2. Anand CR, Linkswiler H. Effect of protein intake on calcium balance of young men given 500 mg calcium daily. J Nutr **104**:695–700, 1974.
3. Allen LH, Hall TE. Calcium metabolism, intestinal calcium-binding protein, and bone growth of rats fed high protein diets. J Nutr **108**:967–972, 1978.
4. Bell RR, Englemann DT, Sie T-L, Draper HH. Effect of a high protein intake on calcium metabolism in the rat. J Nutr **105**:475–483, 1975.
5. Allen LH, Oddoye EA, Margen S. Protein-induced hypercalciuria: a longer term study. Amer J Clin Nutr **32**:741–749, 1979.
6. Allen LH, Bartlett RS, Block GD. Reduction of renal calcium reabsorption in man by consumption of dietary protein. J Nutr **109**:1345–1350, 1979.
7. Howe JC, Beecher GR. Effect of dietary protein and phosphorus levels on calcium and phosphorus metabolism of adult rats. Nutr Reps Int **24**:919–929, 1981.

8. Borer KT, Kuhns LR. Radiographic evidence for acceleration of skeletal growth in adult hamsters by exercise. Growth **41**:1–13, 1977.
9. Kallfelz FA. Correlation between ^{47}Ca absorption and intestinal calcium-binding activity in the golden hamster. Proc Soc Exp Biol Med **139**:77–79, 1972.
10. Biddulph DM. Influence of parathyroid hormone and the kidney on maintenance of blood calcium concentration in the golden hamster. Endocrinology **90**:1113–1118, 1972.
11. Lovelace F, Will L, Sperling G, McCay CM. Teeth, bones and aging of Syrian hamsters. J Gerontol **13**(1):27–31, 1958.
12. National Research Council. Nutrient Requirements of Laboratory Animals. Washington, D.C., Nat Acad Sci, pp70–79.
13. Frier H. Bone growth in the hypo- and hypervitaminotic A male weanling rat. Ph.D. Thesis, University of Connecticut, 1975.
14. Snedecor GW, Cochran WG. Statistical Methods. Ames, Iowa State Univ Press, p272, 1967.
15. Arrington LR, Platt JK, Shirley RL. Protein requirements of growing hamsters. Lab Anim Care **16**:492–496, 1966.
16. Arrington LR, Ammerman CB, Franke DE. Protein requirements of hamsters fed a natural diet. Lab Anim Care **29**:469–476, 1979.
17. LeRoith D, Pimstone BL. Bone metabolism and composition in the protein-deprived rat. Clin Sci **44**:305–319, 1973.
18. Kalk WJ, Pimstone BL. Calcium-binding protein and vitamin D metabolism in experimental protein malnutrition. Brit J Nutr **32**:569–578, 1974.
19. Nordin BE. Osteoporosis. Advan Metab Disord **1**:125–151, 1964.
20. Beecher GR, Coupain JG. Influence of dietary protein and phosphorus during two periods of maturity on bone, blood and muscle of the rat. Fed Proc **38**:872, 1979.
21. Engstrom GW, DeLuca HF. Effect of egg white diets on calcium metabolism in the rat. J Nutr **81**:218–222, 1963.
22. Weiss RE, Gron A, Dux S, Nimni ME. Influence of high protein diets on cartilage and bone formation in rats. J Nutr **111**:804–816, 1981.

Received April 1, 1982. P.S.E.B.M. 1983, Vol. 172.