EFFECT OF PROTEIN SOURCE ON CALCIUM AND MAGNESIUM EXCRETION IN ADULT RATS FED HIGH PROTEIN DIETS

by

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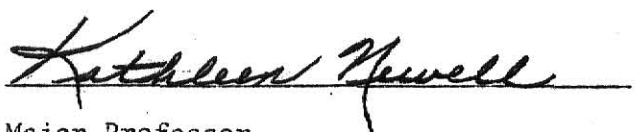

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INTRODUCTION

Osteoporosis is a skeletal disorder in which the absolute amount of bone is decreased relative to that of younger individuals, although the remaining bone is normal in chemical composition (1). Clinically, symptoms include severe backache, pain, progressive loss of height and susceptibility to fractures (2). Symptomatic senile osteoporosis is considered to result from the universal loss of bone that normally attends senescence in both sexes and begins in the third or fourth decade of life (3).

Estimates indicate that a minimum of 10% of the population suffers from senile osteoporosis severe enough to cause vertebral, hip or long-limb fractures (1). The disorder affects one in four women and about half that number of men (4). In general, females have a lower bone mass and this may account, partially, for the higher incidence of osteoporosis among women. The consequences of osteoporosis are magnified in the post-menopausal female, resulting in major orthopedic problems in approximately 25 to 30% of the women in that group (3).

Decreased bone mass may lead to significant incapacitation in aged individuals and result in fractures requiring prolonged hospitalization. Of approximately one million fractures experienced each year by women 45 years of age or older in the United States, about 700,000 are incurred by women with osteoporosis (3). Approximately three-fourths of all deaths from falls occur in patients aged 65 and over, with a female: male fractures incidence ratio of 8:1 (3).

Although the ravages of decreased bone mass are well known, the reasons for age-related skeletal loss is largely conjectural. Heredity,

inactivity, estrogen deficiency and renal dysfunction leading either to inability to conserve calcium or to secondary increments in circulating hormones which resorb bone, i.e. parathyroid hormone, have been implicated as causative factors. Nutritional factors may be involved also, including deficiencies of calcium (5) and/or vitamin D (6); calcium/phosphorous ratio (5); fluoride (7) and acid-ash diets (8).

There is some evidence (5) that a high protein intake is associated with low calcium intake and may play a role in the pathogenesis of osteoporosis. High intakes of dietary protein have been shown to increase urinary excretion of calcium (9, 10), suggesting by inference, that high protein diets decrease the absorption and retention of calcium and thus may be detrimental to bone health. Very little information is available concerning the effect of excess dietary protein on the excretion of other macronutrient elements. Even less is known concerning the effect of protein source on excretion of mineral elements. This study was designed to investigate the effect of excess intake of protein from 3 sources on calcium and magnesium excretion in adult rats fed high protein diets.

REVIEW OF LITERATURE

Effects of Dietary Protein Level on Calcium and Magnesium Excretion

Increased urinary calcium excretion with increased levels of dietary protein has been observed in human beings and laboratory animals for many years. The cause of the hypercalciuria and the source of the increased urinary calcium resulting from high protein diets have yet to be established.

In 1942 McCance et al. (11) observed that increased protein intake by five healthy adults resulted in increased calcium excretion. Hegsted et al. (12) reported in 1952 that a high protein diet increased urinary calcium excretion in adult males and that there was a considerable shift in the distribution of calcium excretion from the feces to the urine.

In 1967, Lindeman et al. (13) observed the effects of various nutrients, including protein, on urinary excretion of divalent cations by healthy male volunteers, ranging in age from 19 to 63 years. Ingestion of glucose, galactose, fructose, protein or ethanol resulted in increased urinary calcium and magnesium, and a decrease in urinary potassium. They reported increased glucose uptake and glycolysis in renal tubule cells following intake of each of those nutrients, and proposed that calcium and magnesium reabsorption and potassium excretion was in some way inhibited in the distal tubules or collecting ducts by the enhanced glucose uptake and glycolysis.

Between 1970 and 1974, Linkswiler and coworkers (9, 14, 15) conducted a series of 3 studies to determine urinary excretion, absorption and retention of calcium in 33 young adult males fed 47.95 or 142g pro-

tein daily at calcium intakes of 500, 800, or 1,400 mg. In each study, protein intakes were varied while calcium intakes were held constant. When the calcium intake was maintained at 1,400 mg increasing the protein intake of 18 to 20 year-old males from 48 to 141 g caused a 163 mg increment in urinary calcium, but an increment of only 69 mg in apparent absorption (9). The level of dietary protein had no significant effect on fecal calcium. At a calcium intake of 800 mg, 19 to 21 year-old male subjects were in positive calcium balance on intakes of 47 and 95 g of protein, but calcium balance was negative on 142 g of protein (14). The lowest protein diet resulted in a significant increase in fecal calcium. All subjects were in negative calcium balance when they were fed diets containing 500 mg calcium and either 95 or 142 g protein (15). Fecal calcium was not affected significantly by protein intake. Based on those studies, Linkswiler et al. (16) concluded that the calcium requirement of the adult human male is influenced by the level of dietary protein.

Margen et al. (10) investigated the calciuretic effect of dietary protein in healthy male volunteers, 20 to 32 years of age. They found a markedly positive correlation between protein intake and urinary calcium excretion. Regardless of calcium intake, varying protein intake from 0 g to 90 g N per day resulted in approximately an 800% increase in calcium excretion, although the response was not linear. A similar calciuretic effect was observed when pure L-amino acid mixtures were substituted for whole protein in the diet.

In a long-term calcium balance study (108 days), Oddoye[1] observed a significant increase in urinary calcium excretion in 6 young male subjects

[1]Oddoye, E. A. (1976) The effect of high and low nitrogen intake on calcium balance. Federation Proc. 35, 499 (Abstr.)

when nitrogen intake was increased from 12 g to 36 g per day. Fecal excretion was slightly higher on the 36 g nitrogen intake than on the 12 g intake. Usually, the subjects were in negative calcium balance during the period of high nitrogen intake. The order of nitrogen feeding affected the degree of negativity, being more negative when the change was from high to low than when the order of feeding was from low to high.

The results of two 51-day metabolic studies of the effect of level of protein and phosphorus on urinary excretion of calcium and calcium balance were reported by Zemel et al.[2] and Hegsted et al.[3] A 4 X 4 Latin square design with 8 subjects in each study was used. In the first study two levels of protein (8 and 24 g N), each with two levels of phosphorus (1,010 and 2,525 mg) were fed. Four diets were fed in the second study: low-protein, low-phosphorus; high-protein, low-phosphorus; and two low-protein diets, one with low-phosphorus and one with high-phosphorus and both with sulfur amino acids to make the sulfur amino acid content equivalent to that in the high-protein diet. Calcium and magnesium levels were maintained at 500 and 350 mg, respectively. There was an approximate doubling of urinary calcium and a substantial decrease in fractional renal tubular reabsorption of calcium in both studies when protein intake was increased. At both levels of phosphorus intake, the high-protein diet caused a decreased calcium balance, attributable to the increased urinary calcium. Urinary calcium was decreased and fractional renal tubular reabsorption of calcium was increased at both levels of protein intake when dietary phosphorus was increased. Adding sulfur amino acids to the low-

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[2]Zemel, M., Schuette, S., Hegsted, M. and Linkswiler, H. M. (1979) Effect of level of protein and phosphorus intake on urinary calcium and sodium in men. Federation Proc. 38, 872 (Abstr.)

[3]Hegsted, M., Schuette, S., Zemel, M. and Linkswiler, H. M. (1979) The effect of level of protein and phosphorus intake on calcium balance in young adult men. Federation Proc. 38, 765 (Abstr.)

protein, low-phosphorus diet resulted in increased urinary excretion, decreased fractional renal tubular reabsorption of calcium and decreased calcium balance, but all the changes were less than those resulting from the high-protein diet. Regardless of the level of protein or sulfur amino acids, high phosphorus intakes resulted in more positive calcium balance than low-phosphorus intakes. The effect of the high protein diet on either urinary calcium or calcium balance was not eliminated by a high level of dietary phosphorus.

Recently, two groups of researchers have investigated the mechanisms involved in the hypercalciuria caused by high levels of protein intake. Linkswiler and her coworkers[4] (17) examined the mechanism of protein induced hypercalciuria by evaluating both renal and parathyroid function in old and young subjects. They conducted a 30 day metabolic study with 5 men (44-86 years) and 6 women (65-79 years). Daily protein intake was maintained at 50 g and 43 g during the first 12 days of the study and 113 g and 110 g during the last 18 days for men and women, respectively. Increased protein intake was accompanied by significant increases in urinary calcium, calcium clearance and net renal acid excretion and significant decrease in tubular reabsorption of calcium. Glomerular filtration rate increased slightly. Protein intake did not affect urinary phosphorus, urinary cyclic AMP, plasma total and ultrafilterable calcium or serum parathyroid hormone. High protein intake resulted in decreased calcium balance. They concluded that the increase in urinary calcium resulted from decreased renal tubular reabsorption and that elderly people exhibit a hypercalciuretic response to increased protein similiar to that observed in young adults.

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[4]Schuette, S A., Zemel, M. B. and Linkswiler, H. M. (1978) Effect of varying protein and sodium intake on calcium metabolism in the elderly. Federation Proc. 37, 892 (Abstr.)

Kim and Linkswiler (17) examined the mechanism of protein-induced hypercalciuria in six young adult males. During a 20-day metabolic study, subjects were fed a 47 g protein diet during the first 10-day period and 142 g during the second 10-day period. Calcium, magnesium and phosphorus intakes were maintained at 515, 320 and 1,110 mg daily, respectively. Increased protein intake resulted in significant increases in urinary calcium, glomerular filtration rate, and calcium clearance and a significant decrease in fractional tubular reabsorption of calcium. Daily urinary magnesium excretion increased significantly when protein intake was increased. Magnesium absorption was elevated slightly and retention was diminished slightly, but neither change was statistically significant. Fasting serum concentration of parathyroid hormone, total calcium, magnesium, inorganic phosphorus and plasma ultrafiltrable calcium were unaffected by the level of protein intake. Their results indicated that protein-induced hypercalciuria may be due to both an increase in glomerular filtration rate and a decrease in fractional renal tubular reabsorption of calcium.

Allen et al. (18) have reported similar findings regarding the mechanism of increased urinary excretion of calcium with increased levels of dietary protein. During a 95-day metabolic study, consisting of two approximately equal periods, six adult male volunteers, between the ages of 23 and 30 years, were fed formula diets supplying 12 g or 36 g nitrogen and approximately 1,400 mg calcium per day. Urinary calcium excretion increased rapidly and significantly from an average of 191 mg/day on the 12 g nitrogen diet to 277 mg/day on the 36 g nitrogen diet. The high intake of dietary protein did not cause significant increases in the apparent absorption of dietary calcium. Overall calcium balance was -37 mg/day and -137 mg/day on the 12 g and 36 g nitrogen diets, respectively. Creatinine

clearance, filtered calcium and urine calcium expressed as percentage of filtered calcium were increased, significantly, by high protein intake, but urine pH, urinary excretion of sodium, potassium, magnesium, phosphorus and hydroxyproline, serum insulin and parathyroid hormone were not increased significantly. They suggested that a decrease in the fractional reabsorption of calcium by the kidney is the most likely cause of protein-induced hypercalciuria, and that consumption of high calcium diets is unlikely to prevent the negative calcium balance and probable bone loss induced by the consumption of high protein diets.

Later, Allen et al. (19), designed a study to further examine the role of the kidney in protein-induced hypercalciuria. Meals containing 18 g and 54 g of protein were fed to each of six male and three female adults, aged 26 to 48 years. The two meals were similar in their energy, sodium, calcium, phosphorus, magnesium and zinc content. Measurements of serum calcium (total and filterable), serum creatinine, and urinary calcium, creatinine, zinc and nitrogen were taken for 4 hours after the meal. Filtered calcium was calculated as the product of glomerular filtration rate, obtained from measurement of serum and urinary creatinine in each clearance period, and serum filterable calcium. Calcium reabsorption was obtained by subtraction of urine calcium from filtered calcium for each clearance period. Between 2 and 4 hours after consumption of the high protein meal, urinary calcium, zinc and nitrogen were significantly higher, but the protein level did not affect urine pH or volume, serum total or filterable calcium or glomerular filtration rate. The percentage of reabsorption of filtered calcium was significantly decreased within 0.5 hour after consumption of the high protein meal, and remained so during the next 3.5 hours. They concluded that high protein consumption lowers the amount of calcium reabsorbed by the kidney.

Bell et al. (20) suggested that the laboratory rat might be a suitable experimental model for studying the mechanism of protein-induced calciuria. They fed adult rats diets containing 10, 20 or 40% protein and uniform concentrations of calcium and phosphorus (0.6 and 0.3%, respectively). Bone resorption was measured by following the excretion of ^{45}Ca which had been incorporated into bone one month before the experimental diet was fed. Rats fed diets containing 40% protein excreted significantly more unlabeled and labeled calcium than animals fed 10 or 20% protein diets. However, fecal ^{45}Ca decreased with increasing dietary protein, and consequently no increase in total ^{45}Ca excretion, an indicator of bone resorption, was observed in the high protein group. High protein intake resulted in enhanced absorption of dietary calcium. They concluded that in the rat, the increased urinary excretion of calcium observed under conditions of high protein intake had no effect on bone resorption when calcium and phosphorus intakes were adequate, and that the increased urinary excretion of calcium was due to a shift in the route of excretion of endogenous calcium from the feces to the urine and to increased absorption of dietary calcium.

Allen and Hall (21) further investigated the use of the rat as an experimental model for protein-induced hypercalciuria. Combined calcium kinetics and balance studies, measurement of intestinal calcium-binding protein activity and bone density fractionation were used to study calcium metabolism in 56-day old male rats, which had been fed an 18% casein (control) or 36% casein (high protein) diet for 2, 14, or 28 days. Urinary calcium excretion was increased 230% by feeding the high protein diet, but there was a tendency for the calciuretic effect of dietary protein to diminish with the length of period fed the high protein diet. No apparent

difference between the urinary calcium excretion of controls and those rats fed the high protein diet for 28 days was observed. Intestinal calcium-binding protein activity was not affected by consumption of the high protein diet for 7 days. Bone density fractionation indicated no change in the rate or extent of bone mineralization attributable to the high protein intake. The investigators pointed out that the daily urine calcium excretion of the rat represents less than 1% of dietary calcium intake, and the increase in urine calcium excretion induced by the high protein diet was very small in comparison to the calcium flux through intestine and bone. They concluded that the rat is not a useful model for studying calcium metabolism in protein-induced calciuria.

Chan et al.[5] tested the hypothesis that the hypercalciuria resulting from high protein intake is associated with the production and urinary excretion of excess acid. They fed 18 male rats diets containing 10, 20, or 60% eggwhite. The calcium, phosphorus and zinc concentrations of the diets were comparable. After 6 weeks, urinary calcium, zinc and net acid excretion were determined and found to be associated with the level of dietary protein. Calcium and zinc excretion were correlated with net acid excretion (r=0.87 and 0.89, respectively) after 6 weeks, and persisted at the 12th week when the analyses were repeated.

In a second experiment, Chan et al.[5] fed rats a 60% eggwhite diet plus 10 meq/kg of $NaHCO_3$ (to "neutralize" the endogenous acid). Urinary calcium and net acid excretion (NAE) were significantly less then during the control period when no $NaHCO_3$ was given, and excretion of calcium and NAE did not differ significantly from that of a group of animals fed the 20% eggwhite diet throughout the experiment. They concluded that zinc excretion, as well as calcium excretion, is affected by the level of pro-

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[5]Chan, W., Calhoun, N. R. and Smith, J. C. (1978) Effect of dietary protein on urinary excretion of calcium and zinc. Federation Proc. 37, 847 (Abstr.)

tein intake, and they suggested that the hypercalciuretic effect of a high protein diet is secondary to the increased acid excretion.

Effects of Dietary Protein Source on Calcium and Magnesium Excretion

It has been suggested (16) that all proteins may not have the same effect on urinary excretion and retention of calcium. Dull[6] studied the effect of dietary gelatin on calcium and magnesium metabolism in human subjects. Complete balance studies were performed on five subjects fed normal diets plus supplemental gelatin. Calcium and magnesium excretions were increased in three subjects fed 50 g of gelatin daily for six days. By the sixth day of gelatin supplementation, urinary calcium increased from average control values of 175, 100 and 82 mg per day to 273, 195 and 141 mg and urinary magnesium increased from 5.5, 4.0 and 7.4 meq per day to 6.5, 6.7 and 9.3. Fecal calcium increased in all three subjects during the six-day period. A negative calcium balance was maintained for 30 days in two subjects consuming gelatin, and in one subject negative calcium balance ranged from 100-215 mg per day during 30 days of gelatin administration. They suggested that the increase in urinary calcium and magnesium was caused by peptide bonding or chelation or peptide inhibition of tubular reabsorption, and that increased endogenous secretion of calcium accounted for the increased fecal calcium.

Whiting and Draper[7] monitored urinary calcium in adult male rats fed an 18% casein control diet (24 g N/kg), or diets containing an additional

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[6]Dull, T. (1963) Effect of dietary gelatin on Ca, Mg and P metabolism. Clin. Res. 11, 404 (Abstr.)

[7]Whiting, S. J. & Draper, H. H. (1978) Studies on the calciuric effect of excess dietary protein. Federation Proc. 37, 847 (Abstr.)

24 g N/kg from lactalbumin (Lact), eggwhite (EW), gelatin (Gel) or casein (Cas). All diets contained 0.6% calcium and 0.3% phosphorus. Urinary calcium excretion reached a peak at two days when the relative rates of excretion expressed as percent of control were: Lact-489; Cas-340 and Gel-263. Calcium excretion decreased to generally stable rates between 4 and 8 weeks with the following average values relative to that of the controls: Lact-201; EW-170; Cas-148 and Gel-135. The calciuretic effects of the high protein diets were in the same order as their total sulfur content, i.e., Lact > EW > Cas > Gel. They suggested that sulfate formed by the catabolism of excess sulfur amino acids is a factor in the calciuretic effect of high protein diets.

Calvo and Bell[8] conducted a 20-week study to determine the long-term effects of various protein sources on calcium metabolism and bone status of 500 g male rats, "deep-labeled" with ^{45}Ca. Control rats were fed diets containing 6% protein as casein and compared to rats fed diets containing 6% casein plus 24% protein as either lactalbumin, beef, casein, soy, egg-white or gelatin. Magnesium, phosphorus and calcium levels in the diets were held constant at 0.18, 0.4 and 0.6%, respectively. Calciuria was not induced by all protein sources. Diets containing lactalbumin, eggwhite and gelatin resulted in greater urinary excretion of ^{45}Ca then proteins in other diets. None of the proteins increased bone resorption or altered femur weight or ash content when calcium intake was adequate.

In a second study, Calvo and Bell[8] investigated the effects of high protein and inadequate calcium intakes on calcium metabolism in adult rats. Control rats fed 10% protein as casein were compared with rats fed 10% casein with protein increased to moderate (25%) or high (40%) levels

[8]Calvo, M. S. & Bell, R. R. (1978) Effect of protein-induced calciuria on calcium metabolism and bone integrity of adult rats. Federation Proc. 37, 891 (Abstr.)

by the addition of both eggwhite and beef. Each level of protein was fed at adequate (0.6%) and inadequate (0.05%) levels of calcium intake. Regardless of protein intake, dry fat-free and ashed femur weights were significantly lower when dietary calcium was inadequate. Their studies indicated that increasing dietary protein induced calciuria in adult rats, but did not increase bone resorption or alter bone composition when calcium intake was either adequate or inadequate.

Benke[9] examined the effect of both level and source of protein on urinary calcium, zinc and titratable acidity. They fed adult male rats complete diets containing 20% protein from lactalbumin (Lac), casein (Cas) and soy isolate (SI) for 2 weeks, followed by a two-week period during which the same rats were fed 40% protein from the same sources. The diets supplied 0.6% Ca and 33 ppm Zn. Urinary calcium (mg/24 hr) and Zn (μg/24 hr) at the 20% protein level were: Lac 1.82, 14.3; Cas 0.77, 4.93; SI 0.72, 3.7. When protein was increased to 40%, urinary calcium and zinc increased significantly. Titratable acidity also increased but not significantly for soy isolate.

Benke[9] also investigated the calciuretic effect of complete diets containing 20% protein from peanut (PN), eggwhite (EW), beef and a 70/30 mixture of beef/soy isolate (SI). The diets contained 0.6% Ca and adequate essential amino acids. Urinary calcium excretion (mg/24 hr) was: PN 0.84, EW 1.11, beef 0.94 and beef/SI 0.55. Titratable acidity did not correlate with urinary calcium in either study. The studies showed the stimulatory effect of high protein diets on urinary calcium and zinc and that different proteins cause different degrees of hypercalciuria. The investigator suggested that high protein-induced hypercalciuria may be a function of the specific dietary protein fed.

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[9]Benke, S. S. (1979) Effect of different protein sources on urinary calcium and zinc in adult male rats. Federation Proc. 38, 872 (Abstr.)

MATERIALS AND METHODS

Animals

Forty male, 5 month-old rats,[1] averaging 350g in weight initially, were used in the study. All animals had been fed Wayne Lab Blox, a commercial stock diet containing 24.5% protein, 4.2% fat, 3.2% fiber, 1.2% calcium, 1.0% phosphorus and other essential minerals and vitamins, since weaning. The rats were housed in individual, wire-bottom, stainless steel metabolism cages with feed and distilled water provided ad libitum during the study. Feed intakes and body weights were recorded weekly and at the beginning and end of each collection period.

Diets

The rats were randomized into replications according to initial body weights. Experimental diets and animals within a replication were assigned at random to individual cages. The composition of the diets is presented in table I. A diet providing 15% protein as casein, was fed to one group of animals for comparison of calcium and magnesium excretion. Three other diets provided 30% protein as casein, casein and lactalbumin or casein and gelatin.

The dietary proteins were analyzed for nitrogen, using a micro-Kjeldahl digestion unit[2] and the AOAC method (22), in the Analytical Services Laboratory, Department of Animal Sciences and Industry. The diets were formulated to provide 15% or 30% protein, based on the nitrogen determinations. All diets contained 10% fat. They were made isocaloric by adjusting the dextrose level.

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[1]Rats of the NLR strain, Wistar origin, purchased from National Laboratory Animals, O'Fallon, Missouri.

[2]Labcono Micro-Kjeldahl Digestion Unit, Kansas City, Missouri.

TABLE I

Percent composition of semisynthetic diets

Component	Dietary treatment			
	15% Casein (10)[1]	30% Casein (10)	30% Casein-lactalbumin (10)	30% Casein-gelatin (10)
Casein, vitamin free[2]	16.55	33.10	16.55	16.55
Lactalbumin[3]	-	-	19.35	-
Gelatin[3]	-	-	-	15.00
Dextrose[2]	66.82	50.30	48.19	51.91
Fat (lard)	8.00	8.00	8.00	8.00
Vegetable oil[4]	2.00	2.00	2.00	2.00
Alphacel[2]	1.50	1.50	1.50	1.50
Vitamin mix[5]	2.00	2.00	2.00	2.00
Mineral mix[6]	0.782	0.782	0.782	0.782
$MgCO_3$	0.14	0.13	0.11	0.12
$CaCO_3$	0.99	0.96	0.82	0.92
$Ca(H_2PO_4)_2 \cdot H_2O$	1.22	1.22	0.68	1.22

[1]The numbers in parentheses refer to the number of animals in each group.

[2]Vitamin-free casein, dextrose, lard and alphacel (nonnutritive bulk), ICN Nutritional Biochemicals, Cleveland, Ohio.

[3]Lactalbumin and gelatin, United States Biochemical Corp., Cleveland, Ohio.

[4]Soybean and cottonseed oils (Wesson).

[5]Vitamin Diet Fortification Mixture, ICN Nutritional Biochemicals, Cleveland, Ohio. Supplying (in mg/100g diet): retinyl acetate, 0.53: ergocalciferol, 0.004; D,L-tocopherol acetate, 10.0; ascorbic acid, 90.0, inositol, 10.0; choline chloride, 150.0; menadione, 4.5; p-aminobenzoate, 10.0; niacin, 9.0; riboflavin, 2.0; pyridoxine·HCl, 2.0; calcium pantothenate, 6.0; biotin, 0.04; folic acid, 0.18; vitamin B_{12}, 0.0026.

[6]Containing (% of diet): $ZnCO_3$, 0.0096; $FeSO_4 \cdot 7H_2O$, 0.0124; $CuSO_4 \cdot 5H_2O$, 0.002; $MnSO_4 \cdot H_2O$, 0.015; KI, 0.00013; NaCl, 0.23; Na_2CO_3, 0.16: K_2CO_3, 0.353.

All diets were calculated to provide 0.04% magnesium as $MgCO_3$ and 0.6% calcium and 0.3% phosphorus as $CaCO_3$ and $Ca(H_2PO_4)_2 \cdot H_2O$. Diet analyses in the Analytical Services Laboratory confirmed the levels of those minerals in the diet. Calcium and magnesium concentrations were measured using an atomic absorption spectrophotometer[3] and modified AOAC methods (22). Phosphorus content was determined spectrophotometrically using the Fiske and Subbarow method (23). The mineral and vitamin mixtures provided the maintenance requirements of those nutrients for adult rats (24).

All diets were analyzed for sulfur content in the Soil Testing Laboratory, Department of Agronomy. The samples were digested with nitric-perchloric acid and the sulfur in the digest was detected with a $BaCl_2$ turbimetric procedure (25, 26) automated for an autoanalyzer[4]. The percent sulfur in the diets was: 15% casein - 0.035; 30% casein - 0.050; 30% casein and gelatin - 0.23 and 30% casein and lactalbumin - 0.84.

Urine and Fecal Collections

Beginning on day 1 of the study, a 96-hour collection of urine and feces was obtained from each rat. Three successive urine and fecal collections were obtained beginning on days 14, 28 and 42.

During collection periods, urine samples were collected every 24 hours in 120 ml, acid-rinsed glass bottles, containing 0.2 ml of a urine preservative, 10%(w/v) solution of thymol in propanol (27). In order to reduce the contamination of the urine samples with feed or feces, the bottle openings were covered with nylon netting. Daily, freshly prepared collection bottles were provided and the funnels of the metabolism cages were washed thoroughly

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[3]Model 82500 MV flame spectrophotometer, Jarrell Ash, Waltham, Massachusetts

[4]Technicon Auto-Analyzer, Technicon Instrument Corporation, Tarrytown, New York.

and rinsed with distilled water. At the end of each 24 hour period, the urine in each collection bottle was transferred to an acid-rinsed polyethylene bottle, covered and refrigerated until the 96-hour collection was completed. Then, the samples were stored at 0° until analyzed.

During collection periods, fecal samples were collected every 24 hours and stored at room temperature in 120 ml, acid-rinsed, wide-mouth glass jars covered with cheesecloth. At the end of each 96-hour collection period, the pooled fecal samples were air-dried at room temperature for 72 hours and then oven dried at 105° for 24 hours. Dried samples were transferred to acid-rinsed, plastic scintillation vials and stored at 0° until analyzed.

Determination of Urinary pH, Volume, Calcium and Magnesium

Prior to storage at 0°, the pH of each 96-hour urine sample was measured using a pH meter equipped with a single probe combination electrode.

Urine samples from each collection period were thawed at room temperature, transferred to acid-rinsed graduated cylinders, and the 96-hour urine volumes were recorded. Each urine sample was transferred to a 120 ml, acid-rinsed coors evaporating dish and charred under heat lamps for 24-48 hours, depending on the volume. Charred urine samples were transferred to a cold muffle furnace and ashed at 550° for 24 hours. Each ashed sample was dissolved in 10 ml of 6N HCl and heated to boiling on a hot-plate. The sides of the evaporating dish were rinsed with demineralized water and the solution was reheated to boiling. The ash solution was transferred to a 50 ml volumetric flask, diluted to volume with demineralized water and then transferred to an acid-rinsed polyethylene bottle. Calcium and magnesium concentrations of the ash solutions were measured using an atomic absorption spectrophotometer[3] and modified AOAC methods (22), in the Analytical Services Laboratory, Department of Animal Science and Industry.

Determination of Fecal Calcium and Magnesium

Fecal samples from each 96-hour collection period were thawed at room temperature, transferred to a 30 ml acid-rinsed Coors crucible, placed in a hot muffle furnace and ashed at 550° for 24 hours. Then, the samples were removed, cooled, moistened with 8-10 drops of concentrated nitric acid and returned to the muffle furnace for 5 hours to digest the organic compounds, completely. Each ashed fecal sample was dissolved in 5 ml of 6N HCl and heated to boiling on a hot plate. The sides of the crucible were washed down with demineralized water and the solution was reheated to boiling. The ash solution was transferred to a 100 ml volumetric flask, diluted to volume with demineralized water and then transferred to acid-rinsed polyethylene bottles. Calcium and magnesium concentrations of the ash solutions were measured in the Analytical Services Laboratory, Department of Animal Science and Industry, using an atomic absorption spectrophotometer[3] and modified AOAC methods (22).

Statistical Analyses

Two way analysis of variance with diet and period as the main effects was performed on the data for each measurement (28). Least significant difference (LSD) at the 5% level of probability was calculated when the F-value was significant.

RESULTS AND DISCUSSION

Feed Intakes and Weight Gains

The analyses of variance for feed intakes and weight gains is presented in table II. There were no significant differences in feed intakes attributable to dietary treatments (table III). Feed intakes (table IV) during collection periods 3 and 4 were not significantly different, but during collection period 1 feed intake was lower ($P < 0.05$) than during collection periods 2, 3, or 4. Feed intake was lower ($P < 0.05$) during collection period 2 than during collection periods 3 and 4.

Table II

Analyses of variance of feed intakes and weight gains

		Mean squares	
Source of variation	df	Feed intake	Weight gain
Diet	3	860.06	243.12*
Rat/diet	36	348.09	79.97
Period	3	283939.52***	8716.56***
Diet X period	9	104.34	113.41
Residual	108	196.11	66.35

***$P < 0.001$ *$P < 0.05$

There were significant differences in weight gains attributable to dietary treatments (table III). Rats fed a diet containing 15% casein had lower ($P < 0.05$) mean weight gains than those fed a diet containing 30% casein and lactalbumin or casein and gelatin. Allen and Hall (21) reported that rats fed a 36% protein diet were heavier than control rats fed an 18% protein diet. No difference in mean weight gain was observed between rats fed a diet containing 15% casein or 30% casein, nor were there differences

in mean weight gains among rats fed a diet containing 30% casein, 30% casein and lactalbumin or 30% casein and gelatin. Temporary feeding problems with two rats fed the 30% casein diet accounted for lower body weights of that group than for rats fed the other 30% protein diets. Weight gains of the animals were lowest ($P < 0.05$) during collection period 1 and highest ($P < 0.05$) during collection period 2. Weight gains during collection period 3 were lower ($P < 0.05$) then during collection period 2, but they were higher ($P < 0.05$) than weight gains during collection periods 1 and 4.

TABLE III
Means and standard errors for dietary treatments - feed intakes and weight gains

Diet	Number	Feed intake	Weight gain
		g	g
15% Casein	10	187.06 ± 2.94^{a1}	25.62 ± 1.42^{a}
30% Casein	10	179.22 ± 2.94^{a}	27.55 ± 1.42^{ab}
30% Casein-lactalbumin	10	182.44 ± 2.94^{a}	30.82 ± 1.42^{b}
30% Casein-gelatin	10	189.59 ± 2.94^{a}	30.42 ± 1.42^{b}

[1]Means in a column sharing a common superscript are not significantly different ($P < 0.05$) using LSD test.

TABLE IV
Means and standard errors for collection periods - feed intake and weight gains

Collection Period	Number	Feed intake	Weight gain
		g	g
1 (days 1-4)	10	60.0 ± 2.21^{a1}	8.4 ± 1.29^{a}
2 (days 14-18)	10	206.2 ± 2.21^{b}	43.5 ± 1.29^{b}
3 (days 28-32)	10	233.7 ± 2.21^{c}	33.5 ± 1.29^{c}
4 (days 42-46)	10	238.4 ± 2.21^{c}	29.0 ± 1.29^{d}

[1]Means in a column sharing a common superscript are not significantly different ($P < 0.05$) using LSD test.

Urinary Variables

The analyses of variance for four urinary variables is presented in table V. When the urinary variables were analyzed, there was interaction between dietary treatments and collection periods for all variables. Therefore, the effects of diet and collection period on all urinary variables were compared.

TABLE V

Analyses of variance for four urinary variables

Source of variation	df	Mean squares			
		Volume	pH	Calcium	Magnesium
Diet	3	4949.53***	0.09*	13.48***	63.12***
Rats/diet	36	281.04	0.03	1.04	2.57
Period	3	1500.49***	0.39***	6.85***	3.60**
Diet X period	9	152.92**	0.03***	0.88**	7.19***
Residual[1]	107	58.04	0.01	6.34	0.85

[1]108 df for calcium ***P < 0.001 **P < 0.01 *P < 0.05

Volume

Urinary volumes were lower ($P < 0.05$) during all collection periods for rats fed a diet containing 15% casein, than for rats fed 30% casein, 30% casein and lactalbumin, or 30% casein and gelatin. During collection period 3, rats fed 30% casein had lower ($P < 0.05$) urinary volumes than rats fed 30% casein and lactalbumin. No significant difference in urinary volume was observed between rats fed 30% casein or 30% casein and gelatin. These findings are similar to those of Bell et al. (20) who reported that rats fed a 40% protein diet excreted at least five times as much urine as those fed a 10% protein diet.

TABLE VI

Means and standard errors for dietary treatments and collection periods - four urinary variables

Diet[1]	Collection Period[2] 1 (days 1-4)	2 (days 14-18)	3 (days 28-32)	4 (days 42-46)
Volume				
15% Casein	$^{a}12.80\pm2.41^{a}$	$^{a}13.50\pm2.41^{a}$	$^{a}14.50\pm2.41^{a}$	$^{a}16.40\pm2.41^{a}$
30% Casein	$^{b}23.75\pm2.41^{a}$	$^{b}34.70\pm2.41^{b}$	$^{b}33.05\pm2.41^{b}$	$^{b}45.60\pm2.41^{c}$
30% Casein-lactalbumin	$^{b}26.20\pm2.41^{a}$	$^{b}36.45\pm2.41^{b}$	$^{c}42.75\pm2.41^{c}$	$^{b}45.15\pm2.41^{c}$
30% Casein-gelatin	$^{b}30.65\pm2.41^{a}$	$^{b}33.45\pm2.41^{ab}$	$^{bc}38.95\pm2.41^{b}$	$^{b}45.74\pm2.58^{c}$
pH				
15% Casein	$^{a}6.29\pm0.03^{a}$	$^{a}6.20\pm0.03^{b}$	$^{ba}6.29\pm0.03^{a}$	$^{ba}6.14\pm0.03^{c}$
30% Casein	$^{b}6.43\pm0.03^{a}$	$^{a}6.29\pm0.03^{b}$	$^{b}6.31\pm0.03^{b}$	$^{a}6.06\pm0.03^{c}$
30% Casein-lactalbumin	$^{a}6.33\pm0.03^{a}$	$^{a}6.22\pm0.03^{b}$	$^{a}6.21\pm0.03^{b}$	$^{b}6.18\pm0.03^{b}$
30% Casein-gelatin	$^{c}6.50\pm0.03^{a}$	$^{a}6.27\pm0.03^{b}$	$^{b}6.36\pm0.03^{c}$	$^{b}6.21\pm0.03^{d}$
Calcium				
15% Casein	$^{a}1.20\pm0.18^{a}$	$^{a}1.49\pm0.18^{ab}$	$^{a}1.77\pm0.18^{b}$	$^{a}1.66\pm0.18^{b}$
30% Casein	$^{ba}1.54\pm0.18^{a}$	$^{cb}2.34\pm0.18^{b}$	$^{b}2.56\pm0.18^{b}$	$^{b}2.54\pm0.18^{b}$
30% Casein-lactalbumin	$^{b}2.03\pm0.18^{a}$	$^{c}2.81\pm0.18^{b}$	$^{c}3.22\pm0.18^{bc}$	$^{c}3.63\pm0.18^{c}$
30% Casein-gelatin	$^{ba}1.71\pm0.18^{a}$	$^{ba}2.04\pm0.18^{a}$	$^{a}1.66\pm0.18^{a}$	$^{b}2.60\pm0.18^{b}$
Magnesium				
15% Casein	$^{a}2.14\pm0.29^{a}$	$^{a}2.11\pm0.31^{a}$	$^{a}3.26\pm0.29^{b}$	$^{a}2.58\pm0.29^{ab}$
30% Casein	$^{a}2.10\pm0.29^{a}$	$^{a}2.08\pm0.29^{a}$	$^{a}2.99\pm0.29^{b}$	$^{a}2.66\pm0.29^{ab}$
30% Casein-lactalbumin	$^{b}5.76\pm0.29^{a}$	$^{a}4.41\pm0.29^{b}$	$^{a}3.31\pm0.29^{c}$	$^{a}3.14\pm0.29^{c}$
30% Casein-gelatin	$^{b}5.62\pm0.29^{a}$	$^{c}5.74\pm0.29^{a}$	$^{b}4.39\pm0.29^{b}$	$^{b}4.33\pm0.29^{b}$

[1]Means in a column sharing a common superscript placed to the left of each mean are not significantly different ($P < 0.05$) using LSD test.

[2]Means in a row sharing a common superscript placed to the right of each mean are not significantly different ($P < 0.05$) using LSD test.

Urinary volume of rats fed a diet containing 15% casein did not differ significantly among collection periods (table VI). Rats fed 30% casein had lower ($P < 0.05$) urinary volumes during collection period 1 than during collection periods 2, 3 or 4. Urinary volumes during collection period 4 were higher ($P < 0.05$) than during collection periods 1, 2 or 3. Rats fed 30% casein and lactalbumin had lower ($P < 0.05$) urinary volumes during collection period 1 than during collection periods 2, 3 or 4. Urinary volumes during collection periods 3 and 4 were higher ($P < 0.05$) than during collection periods 1 and 2. Rats fed 30% casein and gelatin had lower ($P < 0.05$) urinary volumes during collection period 1 than collection periods 3 and 4. Urinary volumes during collection period 4 were higher ($P < 0.05$) than during collection periods 1, 2 or 3.

pH

Urinary pH during collection period 1 was lower ($P < 0.05$) in rats fed 15% casein or 30% casein and lactalbumin than in rats fed 30% casein or 30% casein and gelatin. Rats fed 30% casein had lower ($P < 0.05$) urinary pH than rats fed 30% casein and gelatin. Urinary pH did not differ significantly among rats fed 15% casein or 30% casein and lactalbumin. During collection period 2 urinary pH did not differ significantly among the dietary treatments. Urinary pH during collection period 3 was higher ($P < 0.05$) in rats fed 30% casein or 30% casein and gelatin than for those fed 30% casein and lactalbumin. The urinary pH of rats fed 15% casein did not differ significantly from that of animals fed the other diets. Urinary pH during collection period 4 was higher ($P < 0.05$) in rats fed 30% casein and lactalbumin or 30% casein and gelatin than in rats fed 30% casein. The urinary pH of rats fed 15% casein did not differ significantly from that of those fed the other diets. Bell et al. (20) reported no difference in

urinary pH between rats fed 40% and 20% protein diets. Allen et al. (18) reported no change in urinary pH in male subjects when dietary protein was increased from 12g to 36g N per day.

Urinary pH of rats fed 15% casein was higher ($P < 0.05$) in collection periods 1 and 3 than in collection periods 2 and 4; no significant difference in urinary pH was observed during collection periods 1 and 3. During collection period 4, urinary pH was lower ($P < 0.05$) than during collection periods 1, 2 or 3. Rats fed 30% casein had lower ($P < 0.05$) urinary pH during collection period 1 than during collection periods 2, 3 or 4. During collection period 4 urinary pH was lower ($P < 0.05$) than in collection periods 1, 2 or 3, but did not differ significantly between collection periods 2 and 3. Urinary pH for rats fed 30% casein and lactalbumin was higher ($P < 0.05$) during collection period 1 than during collection periods 2, 3 or 4. Urinary pH did not differ significantly during collection periods 2, 3 or 4. For rats fed 30% casein and gelatin urinary pH decreased ($P < 0.05$) with each successive collection period. During this study the urinary pH had a tendency to decrease from collection period 1 to collection period 4.

Calcium

Urinary calcium excretion was lower ($P < 0.05$) during collection period 1 for rats fed 15% casein, than for those fed 30% casein and lactalbumin. Urinary calcium excretion did not differ significantly for rats fed any of the 30% protein diets. During collection period 2 rats fed diets containing 30% casein or 30% casein and lactalbumin had higher ($P < 0.05$) urinary calcium excretion than rats fed 15% casein. Rats fed 30% casein and lactalbumin had higher ($P < 0.05$) urinary calcium excretion than rats fed 15% casein or 30% casein and gelatin. During collection periods 3 and

4, rats fed 30% casein and lactalbumin had higher ($P < 0.05$) urinary calexcretion than rats fed any other diets. During collection period 3, rats fed 15% casein or 30% casein and gelatin had lower ($P < 0.05$) urinary calcium excretion than rats fed 30% casein or 30% casein and lactalbumin. Rats fed 30% casein had higher ($P < 0.05$) urinary calcium excretion than rats fed 15% casein or 30% casein and gelatin. During collection period 4, rats fed 15% casein had lower ($P < 0.05$) urinary calcium excretion than rats fed any other diet. Allen and Hall (21) reported significantly greater urinary calcium excretion in rats fed a 36% protein diet than for animals fed an 18% diet. Allen et al. (18) and Linkswiler et al. (17) reported that increased protein intake resulted in significant elevation of urinary calcium excretion in male subjects.

Analyses of the diets in this study showed that the 30% casein and lactalbumin had the highest sulfur content followed by the 30% casein and gelatin, 30% casein and 15% casein in that order. Urinary calcium excretion of rats fed 30% casein and lactalbumin tended to be higher than that of other rats fed 30% protein diets during collection periods 1 and 2, and the increased excretion was significantly higher ($P < 0.05$) during the last two collection periods. Whiting and Draper (7) observed increased urinary calcium excretion in rats fed 36% protein as casein and casein supplemented with lactalbumin, gelatin or eggwhite. The calciuric effects of the high protein diets were in the same order as their total sulfur content: i.e., lactalbumin > eggwhite > casein > gelatin. Although the sulfur content of the 30% casein and gelatin diet in this study was higher than that of the 30% casein diet it did not result in higher urinary calcium excretion in the rats. Calvo and Bell[1] investigated the effect of protein source on

[1]Calvo, M. S. & Bell, R. R. (1978) Effect of protein-induced calciuria on calcium metabolism and bone integrity of adult rats. Federation Proc. 37, 891 (Abstr.)

calcium metabolism and observed that urinary calcium excretion was greater for rats fed 32% casein supplemented with lactalbumin, eggwhite, or gelatin than for those fed 32% casein supplemented with beef, soy and casein. Increased urinary calcium excretion in human subjects fed normal diets supplemented with gelatin was reported by Dull[2].

Urinary calcium excretion of rats fed 15% casein was lower ($P < 0.05$) during collection period 1 than during collection periods 3 and 4, but did not differ significantly during collection period 2. Rats fed 30% casein had lower ($P < 0.05$) urinary calcium excretion during collection period 1 than during collection periods 2, 3 or 4. Excretion during collection periods 2, 3 and 4 did not differ significantly. Urinary calcium excretion of rats fed 30% casein and lactalbumin was lower ($P < 0.05$) during collection period 1, not significantly different during collection periods 2 and 3 or during collection periods 3 and 4. Rats fed 30% casein and gelatin had higher ($P < 0.05$) urinary calcium excretion during collection period 4 than during collection periods 1, 2 or 3. Excretion during collection periods 1, 2 and 3 did not differ significantly.

Magnesium

Urinary magnesium excretion was higher ($P < 0.05$) in rats fed 30% casein and lactalbumin and 30% casein and gelatin than in rats fed 15% or 30% casein during collection period 1. There were no significant differences in magnesium excretion of rats fed 15% or 30% casein or of rats fed 30% casein or 30% casein and gelatin. During collection period 2 rats fed 30% cas- and gelatin had higher ($P < 0.05$) urinary magnesium excretion than rats fed

[2]Dull, T. (1963) Effect of dietary gelatin on Ca, Mg and P metabolism. Clin. Res. 11, 404 (Abstr.)

any other diet. Rats fed 30% casein and lactalbumin had higher ($P < 0.05$) urinary magnesium excretion than rats fed 15% or 30% casein, but less ($P < 0.05$) than animals fed 30% casein and gelatin. Urinary magnesium excretion did not differ significantly in rats fed 15% or 30% casein. During collection periods 3 and 4 rats fed 30% casein and gelatin had higher ($P < 0.05$) urinary magnesium excretion than rats fed any other diet. Urinary magnesium excretion did not differ significantly in rats fed 15% or 30% casein or 30% casein and lactalbumin. There was a tendency for rats fed 30% casein and lactalbumin or 30% casein and gelatin diets to excrete more magnesium than rats fed 15% or 30% casein diets. Excretion was significantly higher ($P < 0.05$) for rats fed 30% casein and gelatin during collection periods 2, 3 and 4. Kim and Linkswiler (17) reported a significant increase in mean urinary magnesium excretion in adult males with increased protein intake. Allen et al. (18) observed that urinary magnesium excretion in male subjects was not significantly affected when dietary protein level was increased from 12g to 36g N per day.

Urinary magnesium excretion was lower ($P < 0.05$) during collection periods 1 and 2 than during collection period 3 for rats fed 15% or 30% casein. It did not differ significantly during collection periods 1, 2 or 4. Rats fed 30% casein and lactalbumin had higher ($P < 0.05$) urinary magnesium excretion during collection period 1 than during collection periods 2, 3 and 4. They excreted more magnesium ($P < 0.05$) during collection period 2 than during collection periods 3 and 4. The excretion during the last two collection periods did not differ significantly. Magnesium excretion of rats fed 30% casein and gelatin was higher ($P < 0.05$) during collection periods 1 and 2 than during collection periods 3 and 4. Excretion did not differ significantly during collection periods 1 and 2 or during collection periods

3 and 4. The tendency for magnesium excretion to diminish with time suggests adaption to the high protein diets.

Fecal Excretion

The analyses of variance for fecal excretion of calcium and magnesis presented in table VII.

TABLE VII

Analyses of variance for fecal calcium and magnesium

		Mean squares	
Source of variation	df	Calcium	Magnesium
Diet	3	2677.04	50.60
Rat/diet	36	2424.97	17.69
Period	3	11622.54**	459.80***
Diet X period	9	1759.62	29.53**
Residual	107	2844.43	9.97

***P < 0.001 **P < 0.01

Calcium

There were no significant differences in fecal calcium excretion attributable to dietary treatments (table VII). During collection period 4, there was a higher ($P < 0.05$) level of calcium in the feces than during collection periods 1, 2 or 3 (table VIII). No significant differences in fecal calcium excretion during collection periods 1, 2 or 3 were observed. Kim and Linkswiler (17) observed no change in fecal calcium in male subjects when protein intake was increased.

TABLE VIII

Means and standard errors for collection periods - fecal calcium

Collection period	Number	Fecal calcium mg/96 hr.
Period 1 (days 1-4)	10	298.55±0.43[a1]
Period 2 (days 14-18)	10	297.23±8.59[a]
Period 3 (days 28-32)	10	297.51±8.43[a]
Period 4 (days 42-46)	10	331.90±8.43[b]

[1]Means in a column sharing a common superscript are not significantly different ($P < 0.05$) using LSD test.

Magnesium

When the variables were analyzed there was interaction between dietary treatments and collection periods for fecal magnesium (table IX). During collection periods 1 and 2, no significant differences in fecal magnesium excretion attributable to dietary treatment were observed. During collection period 3, rats fed 15% or 30% casein had higher ($P < 0.05$) fecal magnesium excretion than those fed 30% casein and lactalbumin or 30% casein and gelatin. Rats fed 15% casein had higher ($P < 0.05$) fecal magnesium excretion than rats fed 30% casein and gelatin during collection period 4. Excretion did not differ significantly for rats fed 15% or 30% casein or 30% casein and lactalbumin, nor was it significantly different for animals fed any of the 30% protein diets.

Rats fed diets containing 15% casein, 30% casein, 30% casein and lactalbumin, or 30% casein and gelatin had higher ($P < 0.05$) levels of fecal magnesium excretion during collection period 1 than during collection periods 2, 3 or 4. Rats fed 15% or 30% casein had lower ($P < 0.05$) fecal magnesium excretion during collection period 2 than during collection periods

3 or 4, but no significant difference in fecal magnesium excretion was observed during collection periods 3 and 4. No significant differences in fecal magnesium excretion were observed during collection periods 2, 3 or 4 for rats fed 30% casein and lactalbumin or 30% casein and gelatin.

TABLE IX

Means and standard errors for dietary treatments and collection periods - fecal magnesium

	Collection Period[2]			
	1 (days 1-4)	2 (days 14-18)	3 (days 28-32)	4 (days 42-46)
Diet[1]				
15% Casein	$^{a}20.01\pm1.00^{a}$	$^{a}11.31\pm1.00^{b}$	$^{b}17.14\pm1.00^{c}$	$^{b}17.09\pm1.00^{c}$
30% Casein	$^{a}19.24\pm1.00^{a}$	$^{a}10.94\pm1.00^{b}$	$^{b}15.84\pm1.00^{c}$	$^{ba}14.79\pm1.00^{c}$
30% Casein-lactalbumin	$^{a}19.42\pm1.00^{a}$	$^{a}12.22\pm1.07^{b}$	$^{a}11.85\pm1.00^{b}$	$^{ba}14.40\pm1.00^{b}$
30% Casein-gelatin	$^{a}20.05\pm1.00^{a}$	$^{a}11.92\pm1.00^{b}$	$^{a}10.54\pm1.00^{b}$	$^{a}12.48\pm1.00^{b}$

[1]Means in a column sharing a common superscript placed to the left of each mean are not significantly different ($P < 0.05$) using LSD test.

[2]Means in a row sharing a common superscript placed to the right of each mean are not significantly different ($P < 0.05$) using LSD test.

Calcium

The analysis of variance for calcium balance is presented in table X. Rats fed 30% casein had lower calcium intakes ($P < 0.05$) than rats fed 15% casein or 30% casein and gelatin (table XI). Temporary feeding problems with two rats fed the 30% casein diet accounted for the lower calcium intake of that group. Calcium intakes did not differ significantly for rats

fed 15% casein, 30% casein and lactalbumin or 30% casein and gelatin, nor were they significantly different for rats fed 30% casein or 30% casein and lactalbumin.

TABLE X

Analyses of variance for calcium intake, urinary and fecal calcium and calcium balance

Source of variation	df	Mean squares			
		Calcium intake	Urinary calcium	Fecal calcium	Calcium balance
Diet	3	6769.98*	13.48***	2677.04	6002.49
Rat/diet	36	1855.91	1.04	2424.97	2995.91
Period	3	16932.78***	6.85***	11622.54**	7697.50*
Diet X period	9	1390.73	0.88**	2759.62	1391.37
Residual[1]	108	1309.47	0.34	2844.43	2037.87

[1] 107 df for fecal calcium

***P < 0.001 **P < 0.01 *P < 0.05

Calcium intake was higher (P < 0.05) during collection period 4 than during collection periods 1, 2 or 3. No significant difference in calcium intake was observed during collection periods 2 and 3.

Calcium balance was positive during all collection periods. It was less positive (P < 0.05) during collection period 1 than during collection periods 2 and 3. No significant difference in calcium balance was observed during collection periods 1 and 4 or during collection periods 2, 3 and 4. This is in contrast to the effect of high protein intake on calcium balance in human subjects.

Linkswiler and her co-workers (16) reported that subjects were in negative calcium balance on diets containing 141g of protein and 1400mg of calcium; 142g of protein and 800mg of calcium and 95 or 142g of protein and 500mg of calcium.

TABLE XI

Effects of dietary treatments and collection periods on calcium intakes, urinary and fecal calcium and calcium balance.

	Dietary treatment			
	15% casein	30% casein	30% casein-lactalbumin	30% casein-gelatin
Calcium intake, mg/96 hr	396.52±6.82[a][1,2]	370.65±6.82[b]	383.02±6.82[ab]	398.67±6.82[a]
Urinary calcium, mg/96 hr	1.53±0.092[a]	2.25±0.092[a]	2.92±0.092[a]	2.00±0.092[a]
Fecal calcium, mg/96 hr	318.48±8.43[a]	301.82±8.43[a]	303.68±8.59[a]	301.22±8.43[a]
Calcium balance, mg/96 hr	76.52±7.14[a]	66.58±7.14[a]	74.30±7.29[a]	95.45±7.14[a]
	Collection period			
	1 (days 1-4)	2 (days 14-18)	3 (days 28-32)	4 (days 42-46)
Calcium intake, mg/96 hr	360.08±5.72[c]	387.58±5.72[b][2]	391.26±5.72[b]	409.95±5.72[a]
Urinary calcium, mg/96 hr	1.62±0.092[a]	2.17±0.092[a]	2.31±0.092[a]	2.61±0.092[a]
Fecal calcium, mg/96 hr	298.55±8.43[a]	297.23±8.59[a]	297.51±8.43[a]	331.90±8.43[b]
Calcium balance, mg/96 hr	59.90±7.14[b]	86.06±7.27[a]	91.44±7.14[a]	75.44±7.14[ab]

[1]Values are means ± SE

[2]Means in a row sharing a common superscript are not significantly different ($P < 0.05$) using LSD test.

Magnesium intake and balance

The analyses of variance for magnesium balance is presented in table XII. Rats fed 30% casein had a lower ($P < 0.05$) magnesium intake than rats fed 15% casein or 30% casein and gelatin, because of the temporary feeding problems of two animals in the 30% casein group (table XIII). No significant difference in magnesium intake was observed for rats fed 30% casein or 30% casein and lactalbumin nor for rats fed 15% casein, 30% casein and lactalbumin or 30% casein and gelatin.

TABLE XII

Analyses of variance for magnesium intake, urinary and fecal magnesium and magnesium balance

Source of variation	df	Mean squares			
		Magnesium intake	Urinary magnesium	Fecal magnesium	Magnesium balance
Diet	3	30.21*	63.12***	50.60*	8.00
Rat/diet	36	8.25	2.57	17.69	18.42
Period	3	75.27***	3.60	459.80***	851.48***
Diet X period	9	6.18	7.19***	29.53**	71.63***
Residual[1]	107	5.82	0.85	9.97	8.19

[1] 108 df for magnesium intake; 106 df for magnesium balance

***$P < 0.001$ **$P < 0.01$ *$P < 0.05$

Magnesium intake was lower ($P < 0.05$) during collection period 1 than during collection periods 2, 3 and 4, but was higher ($P < 0.05$) during collection period 4 than during collection periods 1, 2 and 3. No significant difference in intake was observed during collection periods 2 and 3.

TABLE XIII

Means and standard errors for dietary treatments and collection periods - magnesium intake

	Dietary treatment			
	15% casein	30% casein	30% casein-lactalbumin	30% casein-gelatin
Magnesium intake, mg/96 hr	26.44±0.43^{a1}	24.71±0.43^{b}	25.54±0.43^{ab}	26.58±0.43^{a}
Urinary magnesium, mg/96 hr	2.52±0.15^{a}	2.46±0.14^{a}	4.16±0.14^{a}	5.02±0.14^{a}
Fecal magnesium, mg/96 hr	16.39±0.50^{a}	15.20±0.50^{a}	14.47±0.51^{a}	13.75±0.50^{a}
	Collection period			
	1 (days 1-4)	2 (days 14-18)	3 (days 28-32)	4 (days 42-46)
Magnesium intake, mg/96 hr	24.00±0.38^{c}	25.84±0.38^{b}	26.08±0.38^{b}	27.33±0.38^{a}
Urinary magnesium, mg/96 hr	3.91±0.14^{a}	3.59±0.15^{a}	3.49±0.14^{a}	3.19±0.14^{a}
Fecal magnesium, mg/96 hr	19.68±0.50^{a}	11.60±0.51^{a}	13.84±0.50^{a}	14.69±0.50^{a}

[1]Means in a row sharing a common superscript are not significantly different ($P < 0.05$) using LSD test.

When the variables were analyzed there was interaction between dietary treatments and collection periods for magnesium balance. During collection period 1, rats fed 15% and 30% casein diets were in positive magnesium balance with the balance more positive ($P < 0.05$) for animals fed 15% casein than for rats fed any other diet. Rats fed 30% casein and lactalbumin and 30% casein and gelatin were in negative magnesium balance. The balance was not significantly different for rats fed 30% casein and 30% casein and gelatin

or for animals fed 30% casein and lactalbumin or 30% casein and gelatin. During collection period 2, the magnesium balance was more positive ($P < 0.05$) for rats fed 15% casein than 30% casein and lactalbumin and 30% casein and gelatin, but did not differ significantly from the balance for rats fed 30% casein. Magnesium balance did not differ significantly for rats fed any of the 30% protein diets. During collection period 3, the balance for rats fed 30% casein and lactalbumin and 30% casein and gelatin was more positive ($P < 0.05$) than those for rats fed 15% or 30% casein. No significant differences in balance were observed for rats fed 15% and 30% casein or 30% casein and lactalbumin and 30% casein and gelatin. During collection period 4, rats fed 30% casein and gelatin had more positive ($P < 0.05$) magnesium balance than animals fed 15% casein. No significant difference in balance was observed for rats fed 15% and 30% casein and 30% casein and lactalbumin, or for animals fed any of the 30% protein diets.

Rats fed 15% casein had more positive ($P < 0.05$) magnesium balance during collection period 2, than during collection periods 1, 3 or 4, (table XIV). Balance did not differ significantly during collection periods 1, 3 and 4. Magnesium balance for rats fed 30% casein was more positive ($P < 0.05$) during collection periods 2 and 4, than during collection periods 1 and 3. It was less positive ($P < 0.05$) during collection period 1 than during any other collection period. Balance did not differ significantly during collection periods 2 and 4. Rats fed 30% casein and lactalbumin were in more positive magnesium balance ($P < 0.05$) during collection period 3 than during any other collection period. Balance was less positive ($P < 0.05$) during collection period 1 than during collection periods 2, 3 and 4. There was no significant difference in balance during collection periods 2 and 4.

During collection period 1, rats fed 30% casein and gelatin were in less positive ($P < 0.05$) magnesium balance than during any other collection period. Balance during collection periods 2, 3 and 4 did not differ significantly.

TABLE XIV

Means and standard errors for dietary treatments and collection periods - magnesium balance

	Collection period[2]			
	1 (days 1-4)	2 (days 14-18)	3 (days 28-32)	4 (days 42-46)
Diet[1]				
15% Casein	$^{a}3.78\pm0.90^{b}$	$^{a}12.51\pm0.97^{a}$	$^{b}6.10\pm0.90^{b}$	$^{b}7.62\pm0.90^{b}$
30% Casein	$^{b}0.82\pm0.90^{c}$	$^{ba}11.71\pm0.90^{a}$	$^{b}5.91\pm0.90^{b}$	$^{ba}9.74\pm0.90^{a}$
30% Casein-lactalbumin	$^{c}-2.12\pm0.90^{c}$	$^{b}8.84\pm0.97^{b}$	$^{a}11.46\pm0.90^{a}$	$^{ba}9.05\pm0.90^{b}$
30% Casein-gelatin	$^{cb}-0.80\pm0.90^{b}$	$^{b}9.08\pm0.90^{a}$	$^{a}11.54\pm0.90^{a}$	$^{a}11.43\pm0.90^{a}$

[1]Means in a column sharing a common superscript placed to the left of each mean are not significantly different ($P < 0.05$) using LSD test.

[2]Means in a row sharing a common superscript placed to the right of each mean are not significantly different ($P < 0.05$) using LSD test.

The results of this study confirmed the findings of other researchers (20, 21) that high intakes of dietary protein increased urinary excretion of calcium in rats. Similar to the observations of Linkswiler et al. (17) with human subjects, urinary excretion of magnesium was increased in the rats by the high protein diets. Different degrees of hypercalciuria were observed

in the rats when different proteins were fed as has been reported by other investigators.[1,3,4] Unlike the results with human subjects (9,14,15), calcium balance in the rats was unaffected by the level of protein in the diet.

[3]Whiting, S. J. & Draper, H. H. (1978) Studies on the calciuric effect of excess dietary protein. Federation Proc. 37, 847 (Abstr.)

[4]Benke, S. S. (1979) Effect of different protein sources on urinary calcium and zinc in adult male rats. Federation Proc. 38, 872 (Abstr.)

SUMMARY

Increased urinary calcium excretion with increased levels of dietary protein has been observed in human beings and laboratory animals, and it has been suggested that it may be an etiologic factor in human osteoporosis. Recently, investigators have attempted to determine the cause of the hypercalciuria and the source of the increased urinary calcium. Few studies have been conducted to determine the effect of protein source on excretion of other macronutrient elements. This study was designed to investigate the effect of protein source on calcium and magnesium excretion in adult rats fed high protein diets.

Groups of adult male rats were fed diets containing 15% protein as casein or 30% protein as casein, casein and lactalbumin or casein and gelatin. All diets contained 10% fat and the calcium, phosphorus and magnesium intakes were maintained at 0.6, 0.3 and 0.04%, respectively. Four 96-hour collections of urine and feces were obtained from each rat, beginning on days 1, 14, 28, and 42 of the study. Feed intakes were recorded weekly and at the beginning and end of each collection period. Urine volume and pH were measured and the calcium and magnesium contents of the urine and fecal samples were determined by atomic absorption spectrophotometry.

There were no significant differences in feed intake attributable to dietary treatments. Weight gains for rats fed 30% protein diets as casein and lactalbumin or casein and gelatin were higher than those for rats fed 15 or 30% casein diets. Throughout the experiment, urinary volume was higher ($P < 0.05$) for rats fed diets containing 30% protein than for those fed diets with 15% protein. There were some differences ($P < 0.05$) in urinary pH attributable to dietary treatments and periods, but the pattern was not

consistent. During the study, urinary calcium tended to be higher for rats fed 30% protein diets than those fed 15% protein, but the increases were significant ($P < 0.05$) only during the fourth collection period. Rats fed the 30% casein and lactalbumin diet tended to excrete more calcium in the urine than rats fed any other diet. There were no significant differences in fecal calcium excretion attributable to source of dietary protein, and all groups of rats maintained positive calcium balance throughout the study, regardless of protein level in the diet.

Urinary excretion of magnesium tended to be higher for rats fed diets containing 30% protein as casein and lactalbumin or casein and gelatin than those fed 15 or 30% casein diets. Rats fed 30% casein and gelatin excreted more ($P < 0.05$) magnesium in the urine than rats fed any other diet during the last three collection periods. Urinary magnesium of rats fed 30% protein as casein and lactalbumin or casein and gelatin tended to decrease with time. Fecal magnesium excretion was unaffected by the protein level of the diet during the first two collection periods, but excretion tended to decrease in rats fed high protein diets during the last two collection periods. With the exception of rats fed 30% protein as casein and lactalbumin or casein and gelatin, all groups of rats were in positive magnesium balance throughout the experiment, irrespective of dietary protein level.

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REFERENCES

1. Lutwak, L. Osteoporosis - a mineral deficiency disease? J. Am. Diet. Assoc. 44, 173 (1964).

2. Posner, B. M. Nutrition in the Elderly. D. C. Heath and Company, Lexington, Massachusetts, 1979 p. 45.

3. Avioli, L. V. Nutritional Disorders of American Women. (M. Winick, editor), John Wiley & Sons, New York, 1977 p. 99-101.

4. Albanese, A. A. Bone Loss: Cause, Detection and Therapy. A. R. Liss, Inc., New York, 1977 p. ix.

5. Lutwak, L. Periodontal disease, Nutrition in Aging. (M. Winick, editor), John Wiley & Sons, New York, 1976 p. 145.

6. Bullamore, J. R., Gallagher, J. C., Wilkinson, R., Nordan, B. E. C. and Marshall, D. H. Effect of age on calcium absorption. The Lancet 2(7672), 535 (1970).

7. Jowsey, J. Prevention and Treatment of Osteoporosis, Nutrition in Aging. (M.Winick, editor), John Wiley & Sons, New York, 1976 p. 145.

8. Ellis, F. R., Pathol, F. R. C., Holesh, S. and Ellis, J. W. Incidence of osteoporosis in vegetarians and omnivores. Am. J. Clin. Nutr. 25, 555 (1972).

9. Johnson, N. E., Alcantra, E. N. and Linkswiler, H. Effect of level of protein intake on urinary and fecal calcium and calcium retention of young adult males. J. Nutr. 100, 1425 (1970).

10. Margen, S., Chu, J.-Y., Kaufmann, N. A. and Calloway, D. H. Studies in calcium metabolism. I. The calciuretic effect of dietary protein. Am. J. Clin. Nutr. 27, 584 (1974).

11. McCance, R. A., Widdowson, E. M. and Lehmann, H. The effect of protein intake on the absorption of calcium and magnesium. Biochem. J. 36, 686 (1942).

12. Hegsted, D. M., Moscosa, I. and Collozoa, C. A study of the minmum calcium requirements of adult men. J. Nutr. 46, 181 (1952).

13. Lindeman, R. D., Adler, S., Yengst, M. J. and Beard, E. S. Influence of various nutrients on urinary divalent cation excretion. J. Lab Clin. Med. 70, 236 (1967).

14. Walker, R. M. and Linkswiler, H. M. Calcium retention in the adult human male as affected by protein intake. J. Nutr. 102, 1297 (1972).

15. Anand, C. R. and Linkswiler, H. M. Effect of protein intake on calium balance of young men given 500 mg calcium daily. J. Nutr. 104, 695 (1974),

16. Linkswiler, H. M., Joyce, C. L. and Anand, C. R. Calcium retention of young adult males as affected by level of protein and calcium intake. Trans. N. Y. Acad. Sci. 36, 333 (1974).

17. Kim, Y. and Linkswiler, H. M. Effect of level of protein intake on calcium metabolism and on parathyroid and renal function in the adult human male. J. Nutr. 109, 1404 (1979).

18. Allen, L. H., Oddoye, E. A. and Margen, S. Protein-induced hypercalciuria: a longer term study. Am. J. Clin. Nutr. 32, 741 (1979).

19. Allen, L. H. and Bartless, R. S. Reduction of renal calcium reabsorption in man by consumption of dietary protein. J. Nutr. 109, 1345 (1979).

20. Bell, R. R., Engelmann, D. T., Sie, T. and Draper, H. H. Effect of a high protein intake on calcium metabolism in the rat. J. Nutr. 105, 475 (1975).

21. Allen, L. H. and Hall, T. E. Calcium metabolism, intestinal calcium-binding protein, and bone growth of rats fed high protein diets. J. Nutr. 108, 967 (1978).

22. A. O. A. C. Official Methods of Analysis, 12th ed., Washington D. C. (1975).

23. Fiske, C. H. and SubbaRow, Y. The colorometric determination of phosphorus. J. Biol. Chem. 66, 375 (1925).

24. National Academy of Sciences. National Research Council, Recommended Dietary Allowances, 8th ed., Washington, D. C. (1974).

25. Blanchar, R. W., Rehm, G. and Caldwell, A. C. Sulfur in plant material by digestion with nitric and perchloric acids. Soil Science Society of America Proceedings 29, 71 (1965).

26. Chesin, L. and Yien, C. H. Turbimetric determination of available sulfates. Soil Science Society of America Proceedings. 15, 149 (1950).

27. Naftalin, L. and Mitchell, L. R. A new urine preservative. Clin. Chem. Acta. 3, 197 (1958).

28. Snedecor, G. W. and Cochran, W. G. Statistical Methods. The Iowa State University Press, Ames, Iowa (1974).

APPENDIX

TABLE XV

Average daily feed intakes during collection periods

Dietary treatment	Rat no.	Collection period 1 (days 1-4)	2 (days 14-18)	3 (days 28-32)	4 (days 42-46)
		g/day			
15% casein	1	19.5	11.6	17.4	19.1
	2	17.4	17.4	17.4	18.5
	3	18.0	17.5	18.4	18.3
	4	17.9	17.1	18.0	17.7
	5	14.5	16.6	16.9	17.3
	6	15.8	16.9	16.3	16.4
	7	16.1	15.9	16.3	17.2
	8	16.3	17.0	14.2	15.3
	9	12.8	16.1	14.7	15.9
	10	14.0	16.5	15.6	15.9
30% casein	11	16.6	16.6	15.6	15.7
	12	13.8	14.4	17.4	17.7
	13	12.1	14.4	14.4	15.6
	14	17.0	15.8	19.1	17.1
	15	16.0	15.9	15.0	14.3
	16	12.9	14.1	14.6	19.9
	17	15.1	15.1	17.6	18.1
	18	14.8	15.6	17.1	17.7
	19	13.1	17.6	16.4	18.0
	20	7.1	14.9	16.4	17.1
30% casein-lactalbumin	21	14.8	18.1	16.1	13.1
	22	14.3	12.2	16.0	17.4
	23	13.5	15.7	20.4	18.7
	24	14.0	15.1	16.0	17.3
	25	14.6	16.5	17.0	18.6
	26	16.5	17.1	16.0	15.1
	27	14.5	16.4	16.9	16.4
	28	13.6	15.7	15.4	17.1
	29	12.8	16.8	15.3	16.3
	30	15.6	17.9	17.4	17.3
30% casein-gelatin	31	16.3	14.2	17.1	19.4
	32	16.9	20.5	15.0	18.9
	33	15.5	17.9	17.4	19.5
	34	13.1	16.1	17.7	18.8
	35	16.3	16.6	17.1	18.3
	36	15.1	15.2	15.4	15.9
	37	16.5	17.5	16.9	16.9
	38	16.9	15.9	16.4	15.3
	39	14.8	16.2	16.3	18.1
	40	14.0	17.1	16.1	16.4

TABLE XVI

Weight gains or losses during collection periods

Dietary treatment	Rat no.	Days of collection			
		1 (days 1-4)	2 (days 14-18)	3 (days 28-32)	4 (days 42-46)
		g/96 hrs			
15% casein	1	18	25	21	23
	2	11	39	39	25
	3	17	30	42	33
	4	11	47	36	31
	5	0	33	27	27
	6	5	44	30	27
	7	3	37	25	32
	8	17	42	21	15
	9	13	31	27	21
	10	4	36	32	28
30% casein	11	11	40	29	24
	12	3	33	39	37
	13	-1	36	28	27
	14	18	52	49	22
	15	5	47	23	17
	16	6	20	34	55
	17	5	34	41	33
	18	11	53	37	30
	19	2	59	35	15
	20	-11	26	38	40
30% casein-lactalbumin	21	11	41	32	12
	22	6	37	30	34
	23	4	40	40	31
	24	11	42	38	40
	25	11	58	49	36
	26	16	47	30	17
	27	10	51	37	26
	28	18	42	30	34
	29	12	53	31	29
	30	13	63	37	34
30% casein-gelatin	31	18	49	44	41
	32	6	59	12	33
	33	4	49	42	43
	34	-2	59	47	41
	35	18	43	31	26
	36	1	34	23	17
	37	9	54	29	25
	38	6	47	42	16
	39	7	55	35	40
	40	9	52	29	24

TABLE XVII

Urinary pH during collection periods

Dietary treatment	Rat no.	Days of collection			
		1 (days 1-4)	2 (days 14-18)	3 (days 28-32)	4 (days 42-46)
15% casein	1	6.2	6.2	6.3	6.3
	2	6.3	6.2	6.2	5.9
	3	6.4	6.1	6.3	6.2
	4	6.3	6.3	6.4	6.3
	5	6.3	6.2	6.2	6.0
	6	6.3	6.2	6.2	5.9
	7	6.2	6.2	6.2	6.1
	8	6.2	6.2	6.2	6.0
	9	6.6	6.2	6.5	6.5
	10	6.1	6.2	6.4	6.2
30% casein	11	6.3	6.2	6.2	5.9
	12	6.4	6.5	6.5	6.2
	13	6.4	6.2	6.2	6.0
	14	6.5	6.3	6.3	6.1
	15	6.3	6.2	6.2	5.9
	16	6.4	6.2	6.4	6.1
	17	6.4	6.2	6.2	6.0
	18	6.5	6.4	6.4	6.1
	19	6.5	6.3	6.4	6.2
	20	6.6	6.4	6.3	6.1
30% casein-lactalbumin	21	6.3	6.3	6.1	6.2
	22	6.3	6.2	6.3	6.1
	23	6.4	6.2	6.2	6.2
	24	6.4	6.2	6.3	6.2
	25	6.3	6.5	6.3	6.3
	26	6.2	6.1	6.2	6.0
	27	6.4	6.3	6.2	6.2
	28	6.2	6.2	6.1	6.0
	29	6.4	6.1	6.2	6.3
	30	6.4	6.1	6.2	6.3
30% casein-gelatin	31	6.5	6.2	6.2	6.1
	32	6.4	6.2	6.3	6.3
	33	6.5	6.4	6.4	6.3
	34	6.5	6.2	6.3	6.2
	35	6.5	6.2	6.3	6.2
	36	6.5	6.3	6.4	6.2
	37	6.5	6.4	6.4	6.2
	38	6.5	6.2	6.3	6.0
	39	6.5	6.3	6.4	---[1]
	40	6.6	6.3	6.6	6.4

[1]Dash indicates that value was not obtained.

TABLE XVIII

Urinary volumes during collection periods

Dietary treatment	Rat no.	Days of collection 1 (days 1-4)	2 (days 14-18)	3 (days 28-32)	4 (days 42-48)
		ml/96 hrs			
15% casein	1	17.0	9.0	12.0	19.0
	2	13.0	13.5	16.0	13.5
	3	15.0	8.5	13.5	15.0
	4	19.0	24.0	28.0	25.0
	5	20.5	12.0	14.0	10.5
	6	7.0	9.5	9.0	10.0
	7	12.5	11.0	13.0	16.0
	8	8.5	13.0	10.5	11.0
	9	5.5	10.5	19.0	20.0
	10	10.0	24.0	10.0	24.0
30% casein	11	24.0	30.5	11.0	22.0
	12	25.5	40.0	12.0	53.0
	13	22.0	26.0	13.0	33.0
	14	23.0	46.5	14.0	62.0
	15	23.0	29.0	33.0	23.0
	16	22.5	24.0	46.0	45.0
	17	32.0	34.0	47.0	48.0
	18	23.5	29.0	44.0	62.0
	19	33.0	56.0	67.0	62.0
	20	10.0	32.0	43.5	46.0
30% casein - lactalbumin	21	24.0	31.0	32.0	39.0
	22	22.0	31.0	37.0	42.5
	23	19.5	26.5	32.5	34.0
	24	24.5	31.5	36.0	39.0
	25	28.0	60.0	57.5	74.0
	26	31.5	32.0	36.0	31.0
	27	24.5	33.0	42.5	43.0
	28	21.0	30.5	36.0	32.0
	29	28.0	35.0	43.0	42.0
	30	39.0	54.0	75.0	75.0
30% casein - gelatin	31	32.0	29.5	34.0	55.0
	32	34.0	29.0	26.0	35.0
	33	28.5	38.0	54.0	47.0
	34	25.0	30.5	38.5	52.0
	35	28.0	26.0	30.5	36.0
	36	30.0	26.5	29.5	34.0
	37	38.0	41.0	41.0	43.0
	38	28.0	18.0	31.0	34.0
	39	32.0	51.0	49.0	(--)[1]
	40	31.0	45.0	56.0	66.0

[1]Dash indicates that value was not obtained.

TABLE XIX

Calcium intakes during collection periods

Dietary treatment	Rat no.	Days of collection			
		1 (days 1-4)	2 (days 14-18)	3 (days 28-32)	4 (days 42-48)
		mg/96 hr			
15% casein	1	468.0	278.4	418.2	456.0
	2	417.0	418.2	418.2	444.0
	3	432.0	420.0	442.2	438.0
	4	429.0	411.0	432.0	420.0
	5	348.0	397.2	404.4	414.0
	6	378.0	404.4	390.6	390.0
	7	387.0	382.2	390.6	408.0
	8	390.0	408.0	342.6	366.0
	9	306.0	387.6	353.4	378.0
	10	336.0	396.0	382.8	378.0
30% casein	11	399.0	397.8	397.8	372.0
	12	330.0	346.2	346.2	420.0
	13	291.0	344.4	344.4	378.0
	14	408.0	378.6	378.6	408.0
	15	384.0	382.2	382.2	342.0
	16	-----	-----	-----	-----
	17	363.0	363.0	363.0	432.0
	18	354.0	381.0	381.0	420.0
	19	315.0	423.6	423.6	432.0
	20	-----	-----	-----	-----
30% casein-lactalbumin	21	354.0	433.8	385.8	312.0
	22	342.0	293.4	384.0	414.0
	23	324.0	377.4	490.2	444.0
	24	336.0	363.6	384.0	414.0
	25	351.0	396.0	408.0	444.0
	26	396.0	409.8	384.0	360.0
	27	348.0	394.2	404.4	390.0
	28	327.0	377.4	370.2	408.0
	29	306.0	402.6	366.6	390.0
	30	375.0	430.2	416.4	414.0
30% casein-gelatin	31	393.0	339.6	411.0	462.0
	32	405.0	492.0	360.0	450.0
	33	372.0	428.4	416.4	468.0
	34	315.0	387.6	425.4	450.0
	35	390.0	397.8	411.0	438.0
	36	363.0	364.8	370.2	378.0
	37	396.0	420.0	404.4	402.0
	38	405.0	380.4	394.2	366.0
	39	354.0	389.4	390.6	432.0
	40	336.0	411.0	387.6	390.0

[1]Dash indicates values were not obtained.

TABLE XX

Urinary calcium during collection periods

Dietary treatment	Rat no.	Days of collection 1 (days 1-4)	2 (days 14-18)	3 (days 28-32)	4 (days 42-48)
		mg/96 hrs			
15% casein	1	1.43	1.16	0.72	1.84
	2	1.74	1.85	2.33	1.65
	3	1.14	2.04	1.42	1.54
	4	1.72	----[1]	3.23	2.50
	5	1.56	1.13	1.76	0.86
	6	0.82	1.76	1.08	1.18
	7	1.31	2.23	2.13	2.57
	8	0.95	2.18	1.52	1.40
	9	0.63	1.06	1.52	1.18
	10	0.67	1.48	2.03	1.91
30% casein	11	1.72	2.53	2.82	1.82
	12	1.37	1.41	3.92	1.57
	13	1.32	2.68	1.54	2.69
	14	1.80	3.08	2.87	4.26
	15	1.41	2.35	2.61	2.29
	16	2.22	2.33	2.38	2.54
	17	1.91	2.79	2.60	2.65
	18	0.43	2.02	2.46	2.02
	19	1.18	2.27	2.61	2.77
	20	2.09	1.92	1.84	1.82
30% casein-lactalbumin	21	2.35	3.07	2.75	3.95
	22	1.41	2.16	2.14	2.89
	23	2.04	4.19	5.76	5.46
	24	2.05	2.81	3.00	4.59
	25	1.87	2.48	3.01	2.69
	26	2.65	2.55	2.55	2.69
	27	2.04	1.61	2.40	2.34
	28	2.24	3.58	4.44	4.50
	29	1.63	2.26	1.69	2.58
	30	2.00	3.39	4.50	4.63
30% casein-gelatin	31	1.74	1.49	1.06	1.81
	32	2.09	2.27	1.33	3.80
	33	2.54	3.83	3.03	2.88
	34	1.32	1.77	1.40	3.68
	35	2.20	1.52	1.29	2.49
	36	1.49	1.34	1.16	2.40
	37	1.26	1.70	1.69	1.85
	38	1.56	1.10	1.62	2.29
	39	1.42	2.59	1.80	2.32
	40	1.49	2.74	2.24	2.48

[1]Dash indicates values were not obtained.

TABLE XXI

Fecal calcium during collection periods

Dietary treatment	Rat no.	Days of collection 1 (days 1-4)	2 (days 14-18)	3 (days 28-32)	4 (days 42-48)
		mg/96 hrs			
15% casein	1	394.8	225.3	311.2	315.5
	2	364.8	241.5	327.2	336.8
	3	298.1	240.0	337.9	342.8
	4	375.1	299.1	452.6	344.6
	5	243.9	360.0	235.8	331.6
	6	298.1	291.1	372.8	352.7
	7	305.4	283.1	303.6	366.7
	8	334.9	354.7	320.8	245.4
	9	292.0	349.8	306.9	311.4
	10	317.8	391.6	248.2	313.4
30% casein	11	348.5	342.9	263.0	309.3
	12	342.1	288.6	291.8	338.0
	13	253.0	268.6	217.0	287.9
	14	327.6	283.6	328.9	312.8
	15	341.9	275.6	265.8	263.4
	16	324.2	210.5	288.4	362.2
	17	303.8	272.6	314.8	345.5
	18	329.9	336.6	309.4	323.5
	19	265.0	311.4	324.7	382.0
	20	142.4	278.6	311.5	385.6
30% casein-lactalbumin	21	278.7	-----[1]	275.9	301.5
	22	381.8	255.5	312.3	335.4
	23	283.1	270.5	240.0	303.5
	24	152.4	265.2	318.4	340.3
	25	276.6	224.5	216.5	343.6
	26	303.5	298.4	343.4	314.3
	27	318.4	354.0	327.0	298.2
	28	324.3	289.0	305.1	388.7
	29	228.3	344.0	341.0	323.6
	30	303.5	369.3	357.1	367.3
30% casein-gelatin	31	248.5	300.0	259.5	349.0
	32	256.6	395.3	254.3	382.0
	33	251.6	323.5	291.8	335.2
	34	109.5	280.3	291.1	351.7
	35	205.9	279.7	316.6	346.3
	36	360.5	276.1	266.2	311.2
	37	501.4	345.5	280.1	320.2
	38	437.2	231.8	260.3	303.1
	39	283.6	298.5	261.0	354.4
	40	233.4	309.9	250.6	335.2

[1]Dash indicates values were not obtained.

TABLE XXII

Calcium balance during collection periods

Dietary treatment	Rat no.	Days of collection 1 (days 1-4)	2 (days 14-18)	3 (days 28-32)	4 (days 42-48)
			mg/96 hr		
15% casein	1	71.77	51.94	106.28	138.66
	2	50.46	174.85	88.67	105.55
	3	132.76	177.96	102.88	93.66
	4	52.18	111.90	-23.83	72.90
	5	102.54	36.07	166.84	81.54
	6	79.08	111.54	16.72	36.12
	7	80.29	96.87	84.87	38.73
	8	54.15	51.12	20.28	119.20
	9	13.37	36.74	44.98	65.42
	10	17.53	2.92	132.57	62.69
30% casein	11	48.78	52.37	131.98	60.88
	12	-13.47	56.19	50.48	80.43
	13	36.68	73.12	125.86	87.41
	14	78.60	91.92	46.83	90.94
	15	40.69	104.25	113.79	76.31
	16	-----[1]	-----	-----	-----
	17	57.29	87.61	45.60	83.85
	18	23.67	42.38	69.14	93.48
	19	48.82	109.93	96.29	47.23
	20	-----	-----	-----	-----
30% casein lactalbumin	21	72.95	-----	107.15	6.55
	22	-41.21	35.74	69.55	75.71
	23	38.86	102.71	244.44	135.04
	24	181.55	95.59	62.60	69.11
	25	72.53	169.02	188.49	97.71
	26	89.85	108.85	38.05	43.01
	27	27.56	38.59	75.00	89.46
	28	0.46	84.82	60.66	14.80
	29	76.07	56.34	23.91	63.82
	30	69.50	57.51	54.80	42.07
30% casein gelatin	31	142.76	38.11	150.44	111.19
	32	146.31	94.43	104.37	64.20
	33	117.86	101.07	121.57	129.92
	34	204.18	105.53	132.90	94.62
	35	181.90	116.58	93.11	89.21
	36	1.01	87.36	102.84	64.40
	37	-106.66	72.80	122.61	79.95
	38	-33.76	147.50	132.28	60.61
	39	68.98	88.31	127.80	75.28
	40	101.11	98.36	134.76	52.32

[1]Dash indicates values were not obtained.

TABLE XXIII

Magnesium intakes during collection periods

Dietary treatment	Rat no.	Days of collection: 1 (days 1-4)	2 (days 14-18)	3 (days 28-32)	4 (days 42-48)
		mg/96 hr			
15% casein	1	31.20	18.56	27.88	30.40
	2	27.80	27.88	27.88	29.60
	3	28.80	28.00	29.48	29.20
	4	28.60	27.40	28.80	28.00
	5	23.20	26.48	26.96	27.60
	6	25.20	26.96	26.04	26.00
	7	25.80	25.48	26.04	27.20
	8	26.00	27.20	22.84	24.40
	9	20.40	25.84	23.56	25.20
	10	22.40	26.40	25.52	25.20
30% casein	11	26.60	26.52	26.52	24.80
	12	22.00	23.08	23.08	28.00
	13	19.40	22.96	22.96	25.20
	14	27.20	25.24	25.24	27.20
	15	25.60	25.38	25.48	22.80
	16	-----[1]	-----	-----	-----
	17	24.20	24.20	24.20	28.80
	18	23.60	25.40	25.40	28.00
	19	21.00	28.24	28.24	28.80
	20	-----	-----	-----	-----
30% casein lactalbumin	21	23.60	28.92	25.72	20.80
	22	22.80	19.56	25.60	27.60
	23	21.60	25.16	32.68	29.60
	24	22.40	24.24	25.60	27.60
	25	23.40	26.40	27.20	29.60
	26	26.40	27.32	25.60	24.00
	27	23.20	26.28	26.96	26.00
	28	21.80	25.16	24.68	27.20
	29	20.40	26.84	24.44	26.00
	30	25.00	28.68	27.76	27.60
30% casein gelatin	31	26.20	22.64	27.40	30.80
	32	27.00	32.80	24.00	30.00
	33	24.80	28.56	27.76	31.20
	34	21.00	25.84	28.36	30.00
	35	26.00	26.52	27.40	29.20
	36	24.20	24.32	24.68	25.20
	37	26.40	28.00	26.96	26.80
	38	27.00	25.36	26.28	24.40
	39	23.60	25.96	26.04	28.80
	40	22.40	27.40	25.84	26.00

[1] Dash indicates values were not obtained.

TABLE XXIV

Urinary magnesium during collection periods

Dietary treatment	Rat no.	Days of collection 1 (days 1-4)	2 (days 14-18)	3 (days 28-32)	4 (days 42-46)
		mg/96 hrs			
15% casein	1	2.74	1.23	0.84	1.82
	2	2.38	2.07	4.74	3.32
	3	2.64	1.71	2.70	1.76
	4	3.09	----[1]	4.47	2.93
	5	2.33	3.09	4.50	2.20
	6	1.27	1.74	2.94	2.81
	7	2.11	2.76	3.71	3.16
	8	2.08	2.83	2.17	2.12
	9	1.18	0.95	2.21	1.69
	10	1.63	1.78	4.29	3.98
30% casein	11	2.70	2.29	3.04	1.41
	12	1.89	0.89	2.23	3.25
	13	1.88	2.71	3.30	3.06
	14	1.60	1.57	2.40	3.40
	15	2.22	2.90	4.33	3.60
	16	1.86	1.42	1.91	0.97
	17	2.55	3.61	3.43	1.59
	18	1.17	1.63	2.83	3.72
	19	2.12	2.48	3.93	3.14
	20	3.05	1.35	2.50	2.49
30% casein-lactalbumin	21	6.87	6.18	1.95	3.74
	22	1.99	2.47	2.28	2.36
	23	5.28	5.16	3.44	3.15
	24	6.67	3.40	3.20	3.96
	25	7.00	3.99	4.51	1.48
	26	7.35	3.96	2.67	3.71
	27	6.19	2.47	1.85	1.20
	28	6.63	6.05	4.00	3.08
	29	3.81	2.71	2.04	2.95
	30	5.82	7.73	7.14	5.80
30% casein-gelatin	31	5.70	6.35	4.73	4.86
	32	6.57	6.43	3.25	3.44
	33	6.32	4.60	2.31	4.83
	34	4.91	6.09	4.51	3.98
	35	5.55	5.88	2.87	3.72
	36	5.07	5.09	3.27	4.12
	37	6.71	5.44	5.06	5.82
	38	4.76	4.70	4.53	2.82
	39	5.53	6.50	7.43	4.90
	40	5.03	6.37	5.97	4.80

[1]Dash indicates values were not obtained.

TABLE XXV

Fecal magnesium during collection periods

Dietary treatment	Rat no.	Days of collection 1 (days 1-4)	2 (days 14-18)	3 (days 28-32)	4 (days 42-46)
		mg/96 hrs			
15% casein	1	23.70	8.44	18.29	19.60
	2	23.80	9.86	13.30	13.14
	3	17.70	10.67	19.74	22.20
	4	17.70	10.54	21.19	16.71
	5	10.40	12.62	11.16	15.60
	6	18.30	11.37	16.63	11.40
	7	23.80	10.25	17.17	17.84
	8	21.70	13.41	20.05	14.92
	9	20.90	11.83	18.85	20.90
	10	22.10	14.08	15.04	18.55
30% casein	11	23.70	9.96	12.69	10.71
	12	23.10	14.40	19.63	18.70
	13	16.80	8.75	9.61	10.86
	14	21.20	9.56	18.55	14.02
	15	22.10	9.63	12.19	9.75
	16	21.90	9.56	15.87	18.78
	17	18.30	9.97	13.80	14.67
	18	20.70	13.73	20.02	17.21
	19	13.60	13.77	16.83	18.79
	20	11.80	10.11	19.23	14.43
30% casein-lactalbumin	21	16.79	----[1]	10.29	14.67
	22	24.87	13.85	14.15	16.93
	23	19.95	11.03	9.87	14.19
	24	16.68	11.69	11.25	13.48
	25	16.79	10.01	8.71	16.70
	26	20.37	13.80	15.06	14.76
	27	23.48	14.19	14.24	13.56
	28	22.19	9.09	8.89	14.67
	29	12.36	17.23	15.78	14.25
	30	20.71	10.42	10.29	10.82
30% casein-gelatin	31	16.55	10.15	8.38	12.76
	32	23.93	17.29	9.19	15.23
	33	21.11	16.37	13.97	15.67
	34	8.42	8.92	9.16	11.10
	35	11.67	10.00	10.76	13.07
	36	23.58	12.94	13.06	11.64
	37	30.54	14.18	12.18	10.34
	38	30.17	7.37	11.35	11.21
	39	16.91	12.14	8.29	12.75
	40	17.59	9.80	9.09	11.02

[1]Dash indicates values were not obtained.

TABLE XVI

Magnesium balance during collection periods

Dietary treatment	Rat no.	Days of collection 1 (days 1-4)	2 (days 14-18)	3 (days 28-32)	4 (days 42-46)
		mg/96 hr			
15% casein	1	4.76	8.89	8.75	8.98
	2	1.62	15.95	9.84	13.14
	3	8.46	15.62	7.04	5.24
	4	7.81	-----[1]	3.14	8.36
	5	10.47	10.77	11.30	9.80
	6	5.63	13.85	6.47	11.79
	7	-0.11	12.47	5.16	6.20
	8	2.22	10.96	0.62	7.36
	9	-1.68	13.06	2.50	2.61
	10	-1.33	10.54	6.19	2.67
30% casein	11	0.20	14.27	10.79	12.68
	12	-2.99	7.79	1.22	6.05
	13	0.72	11.50	10.05	11.28
	14	4.40	14.11	4.29	9.78
	15	1.28	12.95	8.96	9.45
	16	----	----	----	----
	17	3.35	10.62	6.97	12.54
	18	1.73	10.04	2.55	7.07
	19	5.28	11.99	7.48	6.87
	20	----	----	----	----
30% casein-lactalbumin	21	-0.06	----	13.48	2.39
	22	-4.06	3.24	9.17	8.31
	23	-3.63	8.97	19.37	12.26
	24	-0.95	9.15	11.15	10.16
	25	-0.39	12.40	13.98	11.42
	26	-1.32	9.56	7.87	5.53
	27	-6.47	9.62	10.87	11.24
	28	-7.02	10.02	11.79	9.45
	29	4.23	6.90	6.62	8.80
	30	-1.53	10.53	10.33	10.98
30% casein-gelatin	31	3.95	6.14	14.29	13.18
	32	-3.50	9.08	11.56	11.33
	33	-2.63	7.59	11.48	10.70
	34	7.67	10.83	14.69	14.92
	35	8.78	10.64	13.77	12.41
	36	-4.45	6.29	8.35	9.44
	37	-10.85	8.38	9.72	10.64
	38	-7.93	13.29	10.40	10.37
	39	1.16	7.32	10.32	11.15
	40	-0.22	11.23	10.78	10.18

[1]Dash indicates values were not obtained.

EFFECT OF PROTEIN SOURCE ON CALCIUM AND MAGNESIUM EXCRETION IN ADULT RATS FED HIGH PROTEIN DIETS

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Increased urinary calcium excretion with increased levels of dietary protein has been observed in human beings and laboratory animals, and it has been suggested that it may be an etiologic factor in human osteoporosis. Recently, investigators have attempted to determine the cause of the hypercalciuria and the source of the increased urinary calcium. Few studies have been conducted to determine the effect of protein source on excretion of other macronutrient elements. This study was designed to investigate the effect of protein source on calcium and magnesium excretion in adult rats fed high protein diets.

Groups of adult male rats were fed diets containing 15% protein as casein or 30% protein as casein, casein and lactalbumin or casein and gelatin. All diets contained 10% fat and the calcium, phosphorus and magnesium intakes were maintained at 0.6, 0.3 and 0.04%, respectively. Four 96-hour collections of urine and feces were obtained from each rat, beginning on days 1, 14, 28, and 42 of the study. Feed intakes were recorded weekly and at the beginning and end of each collection period. Urine volume and pH were measured and the calcium and magnesium contents of the urine and fecal samples were determined by atomic absorption spectrophotometry.

There were no significant differences in feed intake attributable to dietary treatments. Weight gains for rats fed 30% protein diets as casein and lactalbumin or casein and gelatin were higher than those for rats fed 15% or 30% casein diets. Throughout the experiment, urinary volume was higher ($P < 0.05$) for rats fed diets containing 30% protein than for those fed diets with 15% protein. There were some differences ($P < 0.05$) in urinary pH attributable to dietary treatments and periods, but the pattern was not

consistent. During the study, urinary calcium tended to be higher for rats fed 30% protein diets than those fed 15% protein, but the increases were significant ($P < 0.05$) only during the fourth collection period. Rats fed the 30% casein and lactalbumin diet tended to excrete more calcium in the urine than rats fed any other diet. There were no significant differences in fecal calcium excretion attributable to source of dietary protein, and all groups of rats maintained positive calcium balance throughout the study, regardless of protein level in the diet.

Urinary excretion of magnesium tended to be higher for rats fed diets containing 30% protein as casein and lactalbumin or casein and gelatin than those fed 15% or 30% casein diets. Rats fed 30% casein and gelatin excreted more ($P < 0.05$) magnesium in the urine than rats fed any other diet during the last three collection periods. Urinary magnesium of rats fed 30% protein as casein and lactalbumin or casein and gelatin tended to decrease with time. Fecal magnesium excretion was unaffected by the protein level of the diet during the first two collection periods, but excretion tended to decrease in rats fed high protein diets during the last two collection periods. With the exception of rats fed 30% protein as casein and lactalbumin or casein and gelatin, all groups of rats were in positive magnesium balance throughout the experiment, irrespective of dietary protein level.