THE EFFECT OF DIETARY PROTEIN ON THE RETENTION OF CALCIUM AND PHOSPHORUS AMONG ANIMALS OF DIFFERENT AGES

A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF MASTER OF SCIENCE

IN THE GRADUATE SCHOOL OF THE

TEXAS WOMAN'S UNIVERSITY

COLLEGE OF NUTRITION, TEXTILES, AND

HUMAN DEVELOPMENT

BY

FANG-CHUOH LIU, B.S.

DENTON, TEXAS

DEC. 1982

The Graduate School

Texas Woman's University

Denton, Texas

December 1982

We hereby recommend that the thesis prepared under our supervision by Fang-Chuoh Liu entitled THE EFFECT OF DIETARY PROTEIN ON THE RETENTION OF CALCIUM AND PHOSPHORUS AMONG ANIMALS OF DIFFERENT AGES

be accepted as fulfilling this part of the requirements for the Degree of Master of Science.

Dissertation/Theses signature page is here.

To protect individuals we have covered their signatures.

ACKNOWLEDGEMENTS

The author wishes to express her sincere appreciation to those who directed and encouraged her throughout this research:

To Dr. George Liepa, chairman of the committee, for his effeots in directing this study; for his supervision and guidance in preparation of this thesis.

To Dr. Alice Milner for her advice and for serving as a member of the author's graduate committee.

To Dr. Nancy Dimarco and Dr. Virginia Brewer for serving as members of the author's graduate committee.

To Mrs. Jessie Ashby for her tolerant advice concerning laboratory techniques.

To Mrs. Myung Park for her consultation and for her aid in the preparation of the computer program for the statistical analysis.

To Mr. and Mrs. Ton-Cheng Liu, the author's parents, for their incouragement, support, and love at all times, thereby helping the author to accomplish her studies at Texas Woman's University.

To Miss Tai-Fen Wei and I-Fen Chen, the author's friends, for their personal assistance.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

REVIEW OF LITERATURE

Supplying the population of the United States and other countries with an adequate amount of protein foods of high quality is an important nutritional concern (1). It is imperative that any dietary proteins do not have a negative effect on mineral metabolism. Presently, there is a concern in America regarding the effect of high intakes of dietary protein on bone metabolism.

Major deficiencies in dietary calcium during skeletal maturation are not overcome during the adolescent growth spurt (2). In general, 1.0 gm per day of calcium with a calcium : phosphorus ratio of 1.5:1 appears satisfactory for bone health (3-5). Since phosphorus is found in many foods and is easily absorbed it is not as likely to be as deficient as calcium. When calcium is absorbed adequately, phosphorus is also absorbed in appropriate amounts (6).

Dietary protein has been shown to alter calcium and phosphorus levels and bone metabolism (7,8). Several mechanisms have been proposed to explain how dietary protein affects bone health. It has been well documented that increasing protein intake leads to an increase in urinary calcium excretion both in experimental animals (9-12) and human subjects (4,7,13-18). This phenomenon has usually been considered as a factor in the development of osteoporosis since the high protein intake not only increases urinary calcium levels but also causes a negative calcium balance (7,17-19). The magnitude and duration of this hypercalciuria has been shown to vary significantly with changes in either the type or amount of dietary proteins. For example, adult rats fed a 40% protein diet consisting of a mixture of gelatin, lactalbumin and casein for 98 days (10) exhibited a 10-fold increase in urinary calcium, lasting at least 78 days, ralative to controls fed 10% casein. In contrast, rats fed 36% casein for 32 days have been reported to exhibit only a slight calciuria which disappeared within 28 days, relative to controls fed an 18% protein diet (9). Since the protein used in the former study was different from that used in the latter study it appears that type of dietary protein may play an important role in regulation of calcium by the body.

Several laboratories have recently investigated causes and mechanisms of protein induced calciuria (12,14, 18). Dietary proteins have been found to differ in their calciuric properties. These differences have been correlated with their sulfur amino acid content (20). Sulfur containing amino acids are oxidized to CO_2, H_2O, urea, sulfate and H^+ ions. Chan (20) has reported that the H^+ ions produced are a major source of endogenous acid. Lemann and his colleagues (21,22) demonstrated that the reabsorption of calcium is inhibited by increased acid production. Several investigators have shown that dietary sulfate is calciuric in research animals (12) and also in human subjects (18,19). They have proposed that this occurs by a decreased renal tubular reabsorption and an increased rate of calcium glomerular filtration. High meat (animal protein) diets are rich in sulfur amino acids. These amino acids have been shown to accelerate the rate of age related bone loss (15 to 20 percent) in carnivorous Arctic Eskimos when compared to U.S. whites (23).

Protein foods of animal origin are usually thought to be of higher quality because of their more satisfactory distribution in the type and amounts of various amino acids present. Since animal foods are expensive and in some countries scarce, it has become necessary to turn to the plant proteins to help alleviate some of the protein-calorie malnutrition found in the world. The use of plant protein for human consumption has been investigated and reviewed in the literature (1,24).

In a study in which bone densities were compared between vegetarians and omnivore controls, the vegetarians were shown to be significantly less prone to osteoporosis (25). The author attributed this phenomenon to the less acid ash intake (i.e., omnivores) of the vegetarians.

Some cereal grains (26) and oilseeds (27) contain substantial amounts of phosphorus in the form of phytic acid (a mixture of inositol polyphosphates). This compound is thought to form insoluble calcium salts or complexes with calcium within the lumen of the intestine, thus causing a serious decrease in the absorption of calcium (28,29). Another concern about phytic acid is the uncertain availability of its phosphorus (30,31). The significance of increased phytic acid consumption on intestinal calcium absorption in both humans and experimental animal· is controversial (29). It is probably because many cereals (28) and oilseeds (32) contain a phytase which hydrolyzes phytic acid during the course of food preparation, and also because the intestinal tract of many species contains a phytate-splitting enzyme which probably is a non-specific alkaline phosphatase (29,33).

Not all of the calcium and phosphorus in foods is absorbed by the body. Approximately 20 to 40 percent of the calcium and 70 percent of the phosphorus consumed by an individual is absorbed from the intestinal tract into the bloodstream. However, the absorption of these two minerals may increase greatly when increased requirements exist such as during periods of rapid bone growth (34). Zornitzer et al. (35) investigated the effect of age on the absorption of calcium by male rats and found that younger animals absorbed and retained more calcium than older animals. Studies regarding calcium absorption in man suggest that it diminishes with age in both sexes, starting at the age of 55-60 years in woman and 65-70 years in man (36). Hoff-Jørgensen (37) studied young puppies and found that the effect of phytic acid on calcium absorption was less pronounced in the younger animals. Different dietary proteins should be examined to determine their effects on the retention of calcium and phosphorus among animals of different ages (immature vs mature).

Objectives

The objectives of this study were:

(1) To compare the effect of dietary animal protein (casein) and plant proteins (soybean protein and cottonseed protein) on the retention of calcium and phosphorus by rats.

(2) To examine a possible role for dietary protein (animal vs vegetable) in age related retention of calcium and phosphorus by rats.

CHAPTER II

MATERIALS AND METHODS

Forty immature (three month old) and forty mature (nine month old) male Fisher 344 rats were obtained for this study. Animals were individually housed in metabolic cages in an air-conditioned room with controlled temperature (20-23°C) and lighting (alternating 12 hour periods of light and darkness). All the animals were fed a pellitized commercial diet (#5001 Purina Lab Chow diet) for three days after arrival. The rats in each age group were divided into three subgroups, and assigned to either a control diet (20% casein used as the source of dietary protein) or one of two experimental diets (20% soybean protein or 20% cottonseed protein used as the source of dietary protein). Both control and experimental diets were isoenergetic and isonitrogenous. The composition of the diets is presented in table 1. Calcium and phosphorus contents of the three diets are shown in table 2. The animals were given food and water ad libitum during the experimental period (28 days).

Feces and urine were collected on a weekly basis for determination of fecal and urinary calcium and phosphorus (appendix I and II). Food consumption and weight gain were

TABLE I

COMPOSITION OF DIETS

INGREDIENT	%
Protein[1]	20.0
PL-methionine	0.3
Corn starch	15.0
Sucrose	50.0
Celulil	5.0
Corn oil[2]	5.0
AIN mineral mixture[3]	3.5
AIN vitamin mixture[4]	1.0
Choline bitartrate	0.2

1. Protein source from either casein, soybean or cottonseed.

2. 0.02% of BHA (antioxidant) was added in the corn oil.

3. The mineral mixture contained (gm/kg mix): $CaHPO_4$, 342.8; NaCl, 74; $(HOC(COOK)\ CH_4COOK)_4.H_2O$, 220.0; K_2SO_4, 52.0; MgO, 24.0; $MgCO_3$, 3.5; KIO_3, 0.01; $FeC_6H_5O_7$, 6.0; $ZnCO_3$, 1.6; $CuCO_3$, 0.3; $(Na_2SeO_3).5H_2O$, 0.01; $CrK(SO_4)_2.12H_2O$, 0.55; sucrose, 118.0.

4. The vitamin mixture contained (mg/kg mix): thiamine, 600; riboflavin, 600; pyridoxine, 700; nicotinic acid, 3; Ca pantothenate, 1.6; folic acid, 200; biotin, 20; Vitamin B-12, 1; Vitamin A, 800; dl-α-tocopheryl, 20; cholecalciferol, 2.5; Vitamin K, 50.

TABLE 2

CALCIUM AND PHOSPHORUS CONTENT OF DIET

	TYPE OF DIETARY PROTEIN		
MINERAL CONTENT	CASEIN	SOYBEAN	COTTONSEED
CALCIUM (%)	0.54	0.52	0.52
PHOSPHORUS (%)	0.50	0.48	0.50
Ca:P	1.08:1.00	1.08:1.00	1.04:1.00

measured every three days. At the end of the experimental period, animals were anesthetized with ether and killed.

Both feces and urine samples were stored at -20°C until analysis could be made.

The weekly fecal samples were homogenized in a blender and aliquots were dry-ashed in a muffle furnace for approximately 14 hours at 600°C. Ash was then dissolved in concentrated nitric acid and diluted to a known volume with distilled water. An aliquot of the diluted ash was removed and analyzed for calcium and phosphorus.

Weekly urine samples were thawed and aliquots were removed and analyzed for calcium and phosphorus in duplicate.

Calcium was measured by atomic absorption spectrophotometry (38). Phosphorus was determined by an automated adaptation of the phosphomolybdic reduction method (39).

Statistical Methods

Data were analyzed using the Student's t-test. The level of significance chosen was $P<0.05$.

CHAPTER III

RESULTS AND DISCUSSION

Effects of Dietary Proteins on Food Intake and Growth

There were no significant differences in dietary intakes when casein (CA), soybean protein (SBP), or cottonseed protein (CSP) diets were compared (Table 3).

Three-month-old rats fed casein diets showed slightly greater weight gains than rats fed either SBP diet or CSP diet. These weight differences were not significantly different however.

Immature rats showed a linear weight gain throughout the experimental period with all diets (Fig. 1). Mature rats initially showed a fairly rapid weight gain and then a decrease in weight with all three diets (Fig. 2).

Effects of Dietary Proteins on Calcium Retention

The retention of calcium and phosphorus was calculated according to the following equation:

Retention = Input (Food Intake) - Output (urine + Feces).

Neither immature nor mature rats showed differences in calcium retention with dietary treatment (Table 4). Urinary and fecal excretion of calcium was not significantly different

TABLE 3

WEIGHT GAIN AND FOOD CONSUMPTION OF 3-MONTH-OLD AND 9-MONTH-OLD RATS

AGE	DIETARY PROTEIN[1]	NO. OF ANIMALS	WEIGHT GAIN (gm/month)	FOOD CONSUMP. (gm/wk)[2]
3 month	CA	12	76	111.7 ± 16.8^{a}
	SBP	13	74	117.4 ± 13.8^{a}
	CSP	13	72	122.7 ± 12.3^{a}
9 month	CA	13	22	103.1 ± 11.2^{a}
	SBP	13	26	106.6 ± 10.7^{a}
	CSP	13	17	110.6 ± 8.8^{a}

1. CA: Casein
   SBP: Soybean
   CSP: Cottonseed

2. $\bar{X} \pm$ Standard deviation. Values in rows with same superscript are not significantly different, $P<0.05$

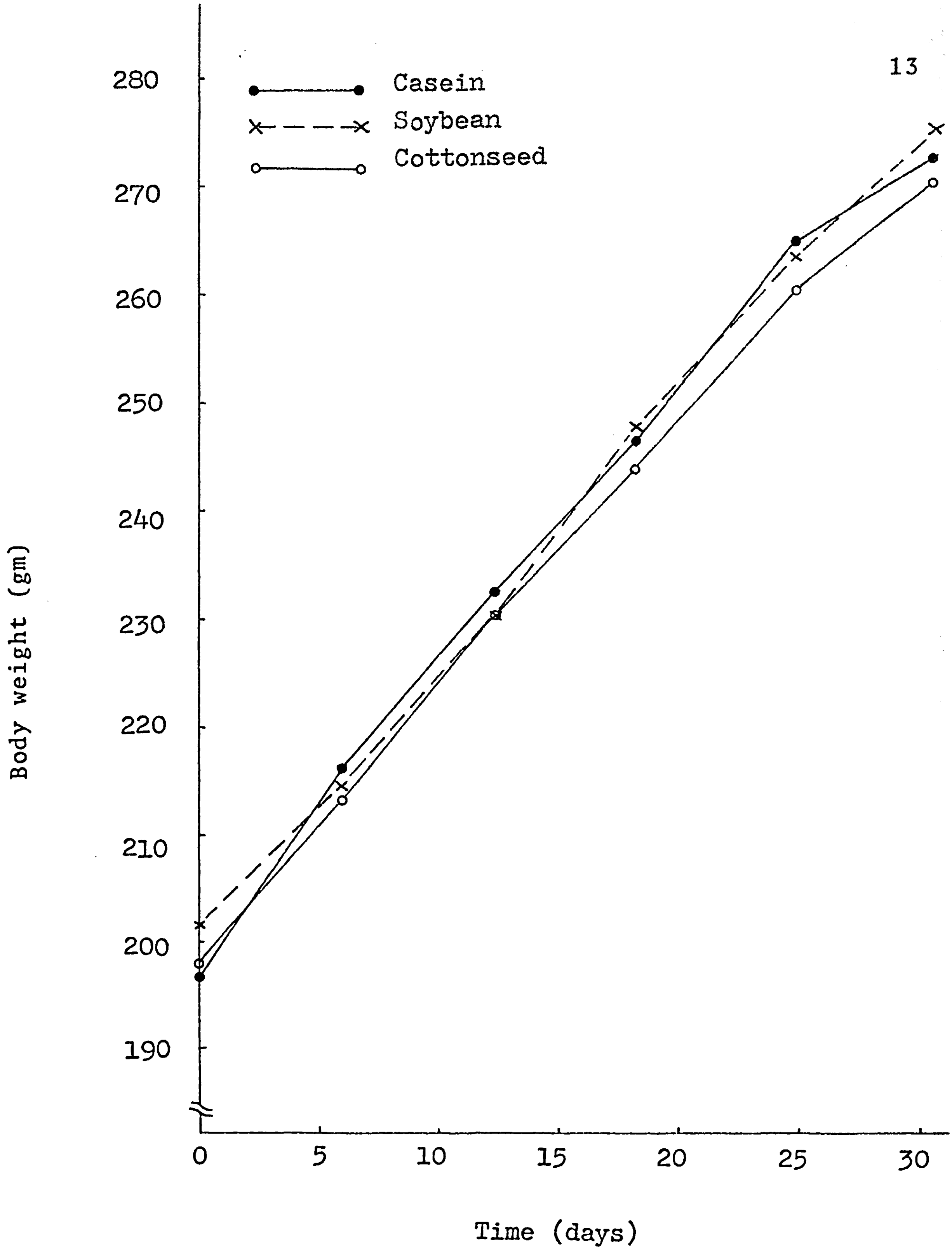

Figure 1 -- Average weight gains of 3-month-old rats fed three different dietary proteins.

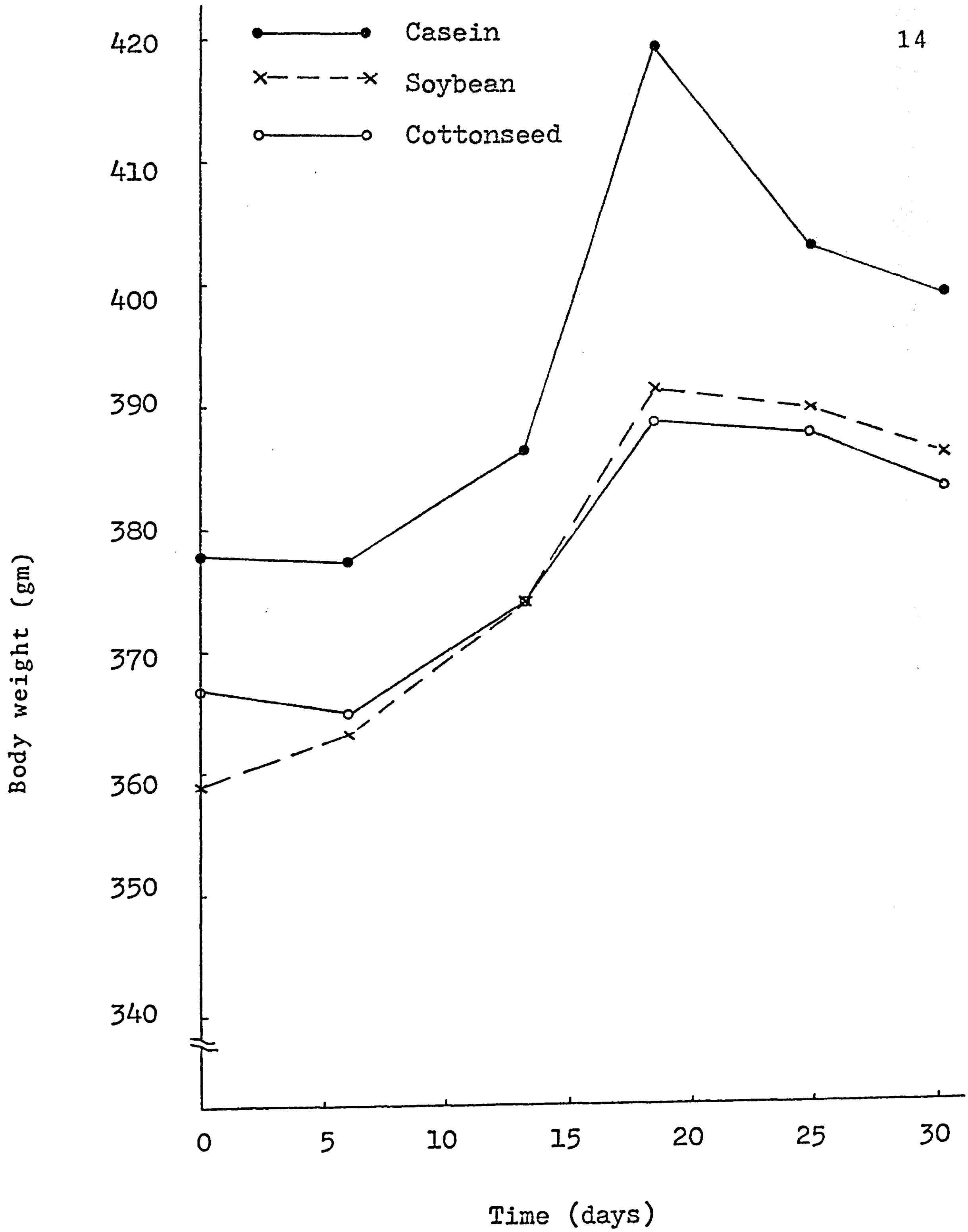

Figure 2-- Average weight gains of 9-month-old rats fed three different dietary proteins.

between animals fed diets containing CA, CSP or SBP. However, significant differences were found in fecal calcium excretion and calcium retention levels between the two age groups.

Some research regarding dietary proteins has involved the feeding of large amounts of protein to experimental animals (9,10) and human subjects (13-15). These studies usually showed significant levels of hypercalciuria. The twenty percent protein diet used in our experiment is considered a moderate level of protein intake when compared to the previously mentioned studies (9,10). When the urinary excretion of calcium in our study was examined, it was much lower than that of animals fed 40% dietary protein in Bell's study(10). Evidence has been obtained (12) showing a role for sulfate, generated by the catabolism of excess sulfur amino acids, in the hypercalciuria of high protein feedings. Casein contains more methinoine and cysteine, which are sulfur-containing amino acids, than either soybean protein or cottonseed protein. However, when examining the effect of different dietary protein sources on urinary calcium excretion (Table 4), the present study showed that there were not significant differences among groups fed either animal protein or vegetable protein. Bell and co-workers

TABLE 4

MEAN INTAKE AND EXCRETION OF CALCIUM OF 3-MONTH-OLD AND 9-MONTH-OLD RATS FED VARIOUS DIETARY PROTEINS

AGE GROUP	DIETARY[1] PROTEIN	Ca[2] CONSUMP. (mg/wk)	URINARY Ca (mg/wk)	FECAL Ca (mg/wk)	TOTAL Ca EXCRETION (mg/wk)	Ca RETENTION (mg/wk)
	CA	605.6±92.7[a]	1.5±0.4[b]	245.1± 72.6[c]	246.6± 72.3[e]	359.6±40.3[g]
3 month	SBP	628.5±74.7[a]	1.3±0.3[b]	269.7±101.8[c]	271.1±101.8[e]	357.4±59.2[g]
	CSP	638.1±75.0[a]	1.6±0.4[b]	272.7± 97.3[c]	274.3± 97.4[e]	364.9±52.4[g]
	CA	557.3±60.4[a]	2.2±0.4[b]	345.3± 52.5[d]	347.5± 58.4[f]	209.8±38.9[h]
9 month	SBP	554.7±55.8[a]	2.0±0.5[b]	348.3± 42.9[d]	350.3± 43.1[f]	204.4±20.9[h]
	CSP	575.4±45.9[a]	2.3±0.6[b]	359.9± 67.1[d]	362.2± 67.0[f]	213.2±29.9[h]

1. CA: Casein
   SBP: Soybean
   CSP: Cottonseed

2. $\bar{X}$±Standard deviation. Values in rows with same superscript are not significantly different, P<0.05.

(10) found that only under stress of acid loads greater than 900 umole/kg of body weight were signs of metabolic acidosis evident. Brosnan et al. (41) reported that adult rats fed a high protein diet (55% casein) developed metabolic acidosis for only 1 to 3 days and that their blood pH and bicarbonate values returned to normal within 7 days. This could explain why our findings did not show higher urinary excretion of calcium by rats fed a 20% casein diet. This diet probably would not develop an acid load in the body and thus would not produce hypercalciuria.

According to data reviewed by Harrison (42), intestinal absorption of calcium decreases between infancy and adulthood. Whereas up to 75 percent of dietary calcium is absorbed in children during periods of skeletal growth, only 30 to 50 percent may be absorbed in adults (43). Johnston (44) observed reduced calcium and nitrogen retention in young girls entering puberty and indicated that sexual maturity altered calcium metabolism. Kon (45) studied changes in the absorption of calcium with age in rats and found that between the age of 4 to 8 weeks, 96 percent of the dietary calcium was absorbed; between 20 to 30 weeks, 25 percent; between 56 to 60 weeks, 15 percent and when they were 93 to 97 weeks old the animals were in negative calcium balance.

Fecal calcium includes that secreted into the gastrointestinal tract as well as unabsorbed calcium(43). When the absorption of calcium decreases, fecal calcium will then increase. Our data indicates that fecal calcium excretion and calcium retention in young male rats is altered significantly with age and perhaps with the onset of sexual maturity. There was significantly lower fecal calcium excretion and higher calcium retention in 3-month-old rats than in 9-month-old rats, regardless of the dietary protein being fed. This is probably because the 3-month-old rats were still growing and thus needed more calcium for bone growth. The 9-month-old rats, on the other hand, had already reached sexual maturity (46), and consequently needed only maintenance levels of dietary calcium.

Effects of Various Dietary Proteins on Phosphorus Retention

When the total excretion and retention of phosphorus of immature rats fed the various dietary proteins were compared (Table 5), differences were not statistically significant.

In mature rats, however, those fed a casein diet showed significantly lower retention of phosphorus than

TABLE 5

MEAN INTAKE AND EXCRETION OF PHOSPHORUS OF 3-MONTH-OLD AND 9-MONTH-OLD RATS FED VARIOUS DIETARY PROTEINS

AGE GROUP	DIETARY PROTEIN[1]	P CONSUMP. (mg/wk)[2]	URINARY P (mg/wk)	FECAL P (mg/wk)	TOTAL P EXCRETION (mg/wk)	P RETENTION (mg/wk)
	CA	568.3±83.1[a]	161.6±12.1[b]	258.6±42.8[c]	420.2±60.3[d]	148.1±26.0[e]
3 month	SBP	563.9±66.3[a]	138.6±16.2[b]	283.9±41.1[c]	422.5±52.8[d]	141.5±32.7[e]
	CSP	598.8±79.2[a]	161.1±15.2[b]	292.3±81.5[c]	453.5±96.5[d]	145.3±57.3[e]
	CA	519.8±53.7[a]	169.8±12.6[b]	290.1±45.0[c]	459.9±85.8[d]	59.9±20.0[f]
9 month	SBP	520.1±49.2[a]	134.1±12.1[b]	295.6±41.1[c]	429.7±67.6[d]	90.3±48.2[g]
	CSP	553.1±44.1[a]	162.6±12.8[b]	297.5±51.0[c]	460.1±70.7[d]	93.0±25.2[g]

1. CA: Casein
   SBP: Soybean
   CSP: Cottonseed

2. $\overline{X}$±Standard deviation. Values in rows with same superscript are not significantly different, $P<0.05$.

rats fed either SBP diets or CSP diets. However, there was no significant difference between groups fed vegetable proteins (SBP vs CSP).

When age-related retention of phosphorus was compared, our data indicated that significant differences existed between immature and mature rats (Table 5).

Little attention has been paid to levels of phosphorus in human diet. This has been a consequence of the fact that the intake of phosphorus is generally higher than the intake of calcium, and thus if the needs for calcium are met, the needs for phosphorus also are met. In addition, increased consumption of meat or processed foods to which phosphorus has been added as a leavening agent, acidifier, or agent for increasing water retention, would tend to increase phosphorus intake (6). When considering the effect of dietary protein on the retention of phosphorus, only certain vegetable protein sources (cereals and oilseeds) are considered to be important. This is because vegetable protein sources contain phytic acid that has significant amounts of phosphorus in it. The absorption of this phosphorus is still questionable. It may combine with calcium and affect the absorption of calcium since calcium phytate is insoluble and excreted in the stools (30,42).

Churella (27) and Nam (47) fed weanling male rats diets containing soybean protein and cottonseed protein for four weeks. These diets were supplemented with varying amounts of calcium and phosphorus. The studies demonstrated that, regardless of the calcium and phosphorus levels of the diet, dietary phytic acid did not interfere with calcium and phosphorus utilization. Our findings also showed no significant differences in calcium and phosphorus retention among the rats fed either vegetable protein or animal protein diets. The low level of fiber in our vegetable protein diets may have significantly altered any possible phytic acid effect. The lower retention of phosphorus by the 9-month-old rats fed casein diets was unexpected.

Another factor which could alter phosphorus metabolism is acid stress. Newell and Beauchene (48) examined the effect of acid stress and age on the renal and bone responses of rats and indicated that although renal adaptations to acid stress occured in both young and old rats, adid-stressed old rats excreted significantly less total acid and phosphorus in the urine than similarly treated young rats. The data from our study showed that the urinary excretion of phosphorus of 9-month-old rats was almont the same as that of the

3-month-old rats. This is probably because any acid stress on our rats would have been marginal considering the moderate levels of protein fed. The decreased phosphorus retention in the mature rats used in this study also could be due to a decreased need for dietary phosphorus in animals requiring minerals primarily for bone maintenance instead of for bone growth (49).

Calcium to Phosphorus Retention Ratio

The calcium to phosphorus retention ratios of immature rats fed CA, SBP and CSP diets were 2.5. The ratios for mature rats fed CA, SBP and CSP diets were 3.5, 2.5 and 2.3 respectively.

Of the calcium retained by the body, from 97 to 98 percent is utilized in the formation of bone; the remainder is found in the body fluids. Stearn (5) indicated that for practical purposes, the retention of calcium can be used as an index of the rate of the growth of bone. Phosphorus, on the other hand, can be utilized by the soft tissues, particularly the muscles, the parenchymatous organs and the nerves. The amount of phosphorus needed for tissues other than bone depends on the rate of growth of these tissues. The actual distribution of the retained phosphorus between the soft tissues and bone is therefore of importance in indicating the comparative rates of growth of the soft and bony tissue. Kramer (50) examined the bone of young rats and concluded that calcium and phosphorus were in an approximate ratio of 2.1:1.0. Stearn (5) indicated that when the retention ratio of calcium to phosphorus was lower than 1.5:1.0, it possibly was indicative of a more rapid growth of soft tissue than of bone. Draper (51) also suggested that a ratio of 2.1:1.0 of calcium to phosphorus is superior to 1:1 for prevention of osteoporosis. The retention ratios of calcium to phosphorus in the present study were all higher than 2.1:1.0 which perhaps indicate that the growth of bone is adequate for these rats. However, the absolute amounts of calcium and phosphorus of the 9-month-old animals were decreasing, which could be expected because the retention of calcium and phosphorus became less as the animals grew older (48).

CHAPTER IV

SUMMARY AND CONCLUSIONS

When the effect of various dietary proteins on calcium and phosphorus retention was examined, no significant difference was found among immature and mature rats. This suggested that neither 20% animal protein (casein) nor 20% vegetable protein (soybean or cottonseed) would affect the retention of calcium and phosphorus by rats.

When calcium and phosphorus retention was examined using animals of different age groups, the mature rats showed significantly less retention of calcium and phosphorus than immature rats. This trend was not affected by the type of protein fed. This might be expected since mature animals would need less calcium and phosphorus as compared to immature, actively growing animals.

APPENDIX

I. Calcium Analysis

A. Urine (38)

1. Weekly samples of urine were thawed at room temperature.
2. Samples were vortexed for a short period of time.
3. Samples were acidified to pH 1 with concentrated HCl.
4. Solution was warmed to 60°C (30 minutes).
   a. Remove 0.1 ml of urine.
   b. Remove 0.1 of Ca standard solution (50 μg Ca/ml, 100 μg Ca/ml, 150 μg Ca/ml).
5. 4.9 ml of 0.1% Lanthanum solution was added.
6. Solution was vortexed for a short period of time.
7. The concentration of Ca was measured using a Atomic Absorption Spectrophotometer (VARIAN AA-475 series).

B. Feces (38)

1. Weekly fecal weight was recorded.
2. Distilled water was added to feces in a 4:1 ratio (Vol:Vol) after an aliquot was removed from weekly samples.
3. Feces was homogenized in a water tight tissue blender.
4. Approximately 0.2 gm of homogenized feces was weighed out.

5. An aliquot of fecal ash was dried in a muffle furnace for 14 hours at 600°C.
6. Ash was dissolved in concentrated nitric acid (0.5 ml) and then diluted to 10.0 ml total volume with distilled water.
7. Solution (0.1 ml) was removed and treated as shown in urinary calcium analysis procedure (steps 4-7).

II. Phosphorus Analysis

A. Urine (39)

1. 9.5 ml of trichloroacetic acid (TCA 5%) was added to 0.5 ml of urine.
2. Samples were vortexed and allowed to stand for 5 minutes.
3. Samples were centrifuged for 20 minutes.
   a. 4.5 ml of TCA (5%) was added to 0.5 ml of filtrate.
   b. 5 ml of TCA (5%) was prepared as a blank.
   c. 5 ml of working standard (0.002 mg P/kg TCA, 0.004 mg P/ml TCA, 0.008 mg P/ml TCA and 0.04 mg P/ml TCA) was prepared as a standard.
4. 1 ml of molybdate reagent was added to a,b, and c.
5. 0.4 ml of aminonaphtholsulfonic acid was added to a,b, and c.
6. 3.6 ml of distilled water was added to a,b,

and c.

8. The concentration of phosphorus was measured by using a spectrophotometer at 690 nm (Perkin-Elmer 552).

B. Feces (39)

Preparation of feces for phosphorus analysis was done as shown in the calcium fecal procedure (steps 1-6). Fecal phosphorus analysis was done according to the procedure shown in the urinary phosphorus technique (steps 1-8).

III Calculations Used to Determine Ca to P Ratio

$$\text{Intake}_{(Ca)} - \text{Urinary}_{(Ca)} - \text{Fecal}_{(Ca)} = \text{Retention}_{(Ca)}$$

$$\text{Intake}_{(P)} - \text{Urinary}_{(P)} - \text{Fecal}_{(P)} = \text{Retention}_{(P)}$$

$$\text{Retention ratio of Ca:P} = \frac{\text{Retention}_{(Ca)}}{\text{Retention}_{(P)}}$$

LIST OF REFERENCES

REFERENCES

1. Bondi, A. Plant Protein: Processed Plant Protein Foodstuff. Edited by A.M. Altschule, pp 43, Academic Press Inc., New York, 1958.

2. Frisancho, A.R., Garn, S.M., and Ascole, W. Childhood retardation resulting in reduction of adult body size due to lesser adolescent skeletal delay. Am. J. Phy. Anthropology 33:325, 1970.

3. Shan, B.G., and Meranger, J.C. Effect of increased dietary phosphorus on calcium metabolism of young rats. Canad. J. Physiol. Pharmacology 48:675, 1970.

4. Spencer, H., Kramer, L., Osis, D., and Norris, C. Effect of phosphorus on the absorption of calcium and on the balance in man. J. Nutr. 108:447, 1978.

5. Stearn, G. The significance of the retention ratio of calcium to phosphorus in infants and children. Am. J. Dis. Child. 42:749, 1931.

6. Renner, R. Nutritional aspects of bone loss. J. Canad. Dietet. Associ. 38:111, 1977.

7. Licata, A.A., Bou, E., Bartter, F.C., and West, F. Acute effects of dietary protein on calcium metabolism in patients with osteoporosis. J. Gerontology 36(1): 14,1981.

8. Hawks, J.E., Bray, M.M., Wilde, M.O., and Dye, M. The interrelationship of calcium, phosphorus and nitrogen in the metabolism of pre-school children. J. Nutr. 24:283, 1942.

9. 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.

10. Bell, R.R., Encelmann, D.T., Sie, T., and Draper, H.H. Effect of high protein intake on calcium metabolism in the rats. J. Nutr. 105:475, 1975.

11. Graves, K.L., and Wolinsky, I. Calcium and phosphorus metabolism in pregnent rats ingesting a high protein diet. J. Nutr. 110:2420, 1980.

12. Whiting, S.J., and Draper, H.H. The role of sulfate in the calciuria of high protein diets in adult rats. J. Nutr. 110:212, 1980.

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

14. Allen, L.H., and Bartlett, R.S. Reduction of renal calcium reabsorption in man by consumption of dietary protein. J. Nutr. 109:1345, 1979.

15. Bleich, H.L. Urinary calcium excretion in human beings. New Engl. J. Med. 301(10):535, 1979.

16. Block, G.D., Wood, R.J., and Allen, L.H. A comparison of the effects of feeding sulfur amino acids and protein on urine calcium in man. Am. J. Clin. Nutr. 33:2128, 1980.

17. Lutz, J., and Linkswiler, M. Calcium metabolism in postmenopausal and osteoporotic women consuming two levels of dietary protein. Am. J. Clin. Nutr. 34:2178, 1981.

18. Schuette, S.A., Zemel, M.B., and Linkswiler, H.M. Studies on the mechanism of protein-induced hypercalciuria in older men and women. J. Nutr. 110:305, 1980.

19. Licata, A.A. Acute effects of increased meat protein on urinary electrolytes and cyclic adenosine monophosphate and serum parathyroid hormone. Am. J. Clin. Nutr. 34:1779, 1981.

20. Chan, J.C.M. The influence of dietary intake on endogenous acid production. Nutr. Metab. 16:1, 1974.

21. Lemann, J., and Relman, A.S. The relation of sulfur metabolism to acid-base balance and electrolyte excretion: the effect of DL-methionine in normal man. J. Clin. Invest. 38:2215, 1959.

22. Lemann, J., Litzow, J.R., and Lennon, E.J. Studies of the mechanism by which chronic metabolic acidosis augments urinary calcium excretion in man. J. Clin.

Invest. 46:1318, 1967.

23. Mazess, R.B., and Mather, W. Bone mineral content of North Alaskan Eskimos. Am. J. Clin. Nutr. 27:1916, 1974.

24. Ziemba, J.V. Cottonseed proteins enter new era. Food Engineer. 44:70, 1972.

25. 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.

26. Cullumbine, H., Basnayake, V., Lemottee, J., and Wickramanayake, T.W. Mineral metabolism on rice diets. Bri. J. Nutr. 4:101,1950.

27. Churella, H.R., and Vivian, V. The effect of phytic acid in soy infant formulas on the availability of minerals for the rat. Fed. Proce. 35:744, 1976.

28. McCance, R.A., and Widdowson, E.M. Phytic acid. Bri. J. Nutr. 2:401, 1949.

29. Wills, M.R. Intestinal absorption of calcium. Lancet 1:820, 1973.

30. McCance, R.A., and Widdowson, E.M. Phytin in human nutrition. Biochem. J. 29:2694, 1935.

31. Taylor, T.C. The availability of the calcium and phosphorus of plant materials for animals. Nutr. Soc. Proce. 24:105, 1965.

32. Ranhotra, G.S., Loewe, R.J., and Puyat, L.V. Phytic acid in soy and its hydrolysis during bread-making. J. Food Sci. 39:1023, 1974.

33. Nelson, T.S. The utilization of phytate phosphorus by poultry- a review. Poultry Sci. 46:862, 1967.

34. Mitchell, H.S., Rynbergen, H.J., Anderson, L., and Dibble, M.V. Nutrition in Health and Disease. pp 55, J.B. Lippincott Co. Philadephia 1976.

35. Zornitzer, A.E., and Bronner, F. In situ studies of calcium absorption in rats. Am. J. Physio. 220:

1261, 1971

36. Bullamore, J.R., Gallagher, J.C., Wildinson, R., and Nordin, B.E.C. Effect of age on calcium absorption. Lancet 2:535, 1970.

37. Hoff-Jøgensen, E. The influence of phytic acid on the absorption of calcium and phosphorus. Biochem. J. 40:189, 1946.

38. Winkelman, J.W., Henry, R.J., and Cannon, D.C. Clinical Chemistry. Principles and Techniques. 2nd ed. pp 637, 1974.

39. Fiske, C.H., and Subbarow, Y. Determination in inorganic phosphate in serum and urine. J. Biol. Chem. 66:375, 1925.

40. Stanley, J.C., and Glass, G.V. Statistical methods in education and psychology. Prentice-Hall Inc. New Jersey, 1970.

41. Brosnan, J.T., McPhee, P., Hall, B., and Parry, D.M. Renal glutamine metabolism in rats fed high-protein diets Am. J. Physiol. 235:E261, 1978.

42. Harrison, H.E. Factors influencing calcium absorption. Fed. Proce. 18:1085, 1959.

43. Avioli, L.V. Intestinal absorption of calcium. Arch. Intern. Med. 129:345, 1972.

44. Johnston, J.A. Factors influencing retention of nitrogen and calcium in period of growth. Am. J. Dis. Child. 59:287, 1940.

45. Kon, E. Milk in relation to human nutrition. J. Roy. Soc. Arts. 93:122, 1944.

46. Porter, G., and Lane-Petter, W. Notes for breeders of common laboratory animals. Academic Press, New York 1962.

47. Nam, S.J. The effects of glandless cottonseed flour on calcium and phosphorus retention in the rats. Master's thesis, Texas Woman's University, 1977.

48. Newell, G.K., and Beauchene, R.E. Effects of dietary calcium level, acid stress and age on renal, serum, and bone responses of rats. J. Nutr. 105:1039, 1975.

49. Henry, K.M., and Kon, S.K. The effect of age and of the supply of phosphorus on the assimilation of the rat. Biochem. J. 41:168, 1947.

50. Kramer, B., and Shear, M.J. Composition of bone. Primary calcification. J. Biol. Chem. 79:147,1928.

51. Draper, H.H., Sie, T., and Bergan, J.C. Osteoporosis in aging rats induced by high phosphorus diet. J. Nutr. 102:1133, 1972.