Report

Mineral Metabolism of Aging Female Rats Fed Various Commercially Available Calcium Supplements or Yogurt

Alison R. Behling[1] and Janet L. Greger[1,2]

Received November 23, 1987; accepted February 17, 1988

The utilization of calcium from commercially available calcium supplements and yogurt and the effects of these calcium supplements on the utilization of other minerals were evaluated. Moderate and high levels (4 and 8 mg Ca/g diet) of calcium from four different sources of dietary calcium (yogurt, calcium phosphate dibasic, calcium magnesium chelate, and oyster shells) were fed to retired female breeder rats. Rats absorbed calcium equally efficiently from all four sources but ingestion of calcium phosphate dibasic tended to cause abnormal accumulation of calcium in kidneys. Ingestion of the calcium magnesium chelate improved calcium retention in bone but depressed the digestibility of the total diet. The elevation of dietary calcium did not affect tissue calcium levels or fecal β-glucuronidase activity but depressed the apparent absorption of phosphorus, increased kidney phosphorus levels, decreased tibia iron levels, and decreased the digestibility of the total diet.

KEY WORDS: calcium; supplements; nephrocalcinosis; magnesium.

INTRODUCTION

The Consensus Development Panel on Osteoporosis (1) suggested that one strategy for preventing or at least slowing the development of osteoporosis was the ingestion of 1000 to 1500 mg calcium daily. Other investigators have suggested that the consumption of additional calcium was protective against colon cancer (2,3). One result of this advice was that sales of calcium supplements increased five-fold between 1980 and 1985 (4).

However, few data are available on the relative utilization of calcium from supplements. Most investigators have examined the utilization of calcium from supplements or foods consumed in a single meal (5–7) but women are advised to consume supplements daily for a number of years. Moreover, the effects of chronic use of calcium supplementation on the utilization of other minerals have been studied primarily with young male rats (8).

Thus the objectives of this study were (i) to compare the utilization of calcium from a dairy product (yogurt) and three commercially available calcium supplements in mature female rats, (ii) to compare the effects of moderate and high calcium intakes on the utilization of calcium, and (iii) to examine the effects of the calcium source and level on the utilization of other essential minerals (i.e., magnesium, iron, phosphorus, zinc, and copper) and on gut function and flora.

MATERIALS AND METHODS

Forty-eight retired female breeder rats were fed one of eight dietary treatments for 33 days. The dietary treatments differed in their levels of calcium (4 and 8 mg Ca/g diet) and in their sources of calcium. Diets 4 yogurt and 8 yogurt contained 4 and 8 mg Ca/g diet, respectively, from freeze-dried yogurt (The Dannon Company, White Plains, N.Y.); Diets 4 oyster shell and 8 oyster shell contained 4 and 8 mg Ca/g diet, respectively, from oyster shells (OsCal 500, Marion Laboratories, Inc., Kansas City, Mo.); Diets 4 Ca Mg chelate and 8 Ca Mg chelate contained 4 and 8 mg Ca/g diet, respectively, from a yeast chelated calcium and magnesium preparation (Schiff Bio-Food Products Manufacturing, Moonachie, N.J.); and Diets 4 $CAHPO_4$ and 8 $CAHPO_4$ contained 4 and 8 mg Ca/g diet, respectively, from calcium phosphate dibasic (Fisher Scientific, Fair Lawns, N.J.).

The semipurified diets fed conformed to the guidelines of the American Institute of Nutrition (9) and were similar in formulation to those fed to weanling rats in previous studies (8). The sole source of calcium in each diet was either yogurt or one of the three supplements because all calcium was eliminated from the basic AIN-76 mineral mixture used to prepare the diets. Diets were formulated to provide 18% protein from either yogurt and/or casein and to provide 9.5% disaccharide from yogurt or sucrose except for Diet 8 yogurt, which contained approximately 19% disaccharide and 23% protein because of the protein and disaccharide content of yogurt. All diets also contained 5.0% cellulose (Teklad Test Diets, Madison, Wis.), 5% corn oil (Best Foods, Englewood Cliffs, N.J.), 3.5% AIN-76 mineral mixture without calcium, 1.0% AIN-76 vitamin mixture (Teklad Test Diets), 0.3% *dl*-methionine, 0.2% choline bitartrate, and cornstarch to achieve 100%. The analyzed composition of the diets is shown in Table I.

Retired female breeder Sprague–Dawley rats (Harlan Sprague–Dawley, Indianapolis, Ind.) were housed individu-

[1] Department of Nutritional Sciences, 1415 Linden Drive, University of Wisconsin, Madison, Wisconsin 53706.
[2] To whom correspondence should be addressed.

0724-8741/88/0800-0501$06.00/0

Table I. Analyzed Content of Rat Diets

Diet	Calcium (mg/g)	Phosphorus (mg/g)	Magnesium (mg/g)	Iron (μg/g)	Zinc (μg/g)	Lactose (%)
4 yogurt	3.96	7.57	0.81	41	31	9.12
8 yogurt	7.52	10.50	1.16	46	40	19.26
4 $CaHPO_4$	4.38	8.92	0.45	45	24	ND[a]
8 $CaHPO_4$	8.18	12.00	0.43	57	25	ND
4 Ca Mg chelate	4.23	5.87	3.11	46	25	ND
8 Ca Mg chelate	8.05	6.83	6.40	55	29	ND
4 oyster shell	3.48	5.90	0.49	43	25	ND
8 oyster shell	7.12	5.56	0.51	53	25	ND

[a] None detected.

ally in stainless-steel wire-bottomed cages. The facilities met the standards of the American Association for Accreditation of Laboratory Animal Care.

Deionized water was offered ad libitum. Food consumption was recorded daily and animals were pair-fed to maintain feed intake at similar levels (11.0 ± 0.1 g feed/day). Rats were weighed twice a week.

Sample Collection and Analyses

Fecal samples were collected on days 8–9 and on days 31–32 of the study. Percentage dry matter digestibility was determined by the following equation: (weight of food intake − weight of dry feces) × 100 ÷ weight food intake. Percentage apparent absorption of minerals was determined by the following equation: (intake of mineral − fecal loss of mineral) × 100 ÷ intake of mineral.

Rats were anesthetized and killed by exsanguination on day 34 of the study after being fasted overnight. Kidneys, livers, and tibias were removed and frozen in acid-washed plastic containers. Fecal pellets were expressed from each rat's colon; β-glucuronidase activity using phenolphthalein glucuronide (Sigma Chemical Company, St. Louis, Mo.) and protein content of diluted fecal pellets were determined (10,11).

Diets, tissues, and fecal samples were analyzed for calcium, magnesium, iron, zinc, and copper by atomic absorption spectroscopy and for phosphorus content by a colorimetric procedure (12,13). Livers were analyzed for iron and copper only; diets were also analyzed for lactose (14). Bovine liver standards (SRM No. 1577a) or milk standards (SRM No. 1549) obtained from the National Bureau of Standards were analyzed with several batches of experimental samples. Liver standards ($N \geq 24$) were determined to contain 600 ± 6 (mean ± SE) μg Mg/g (certified value, 600 μg Mg/g), 123 ± 1 μg Zn/g (certified value, 123 μg Zn/g), 177 ± 4 μg Fe/g (certified value, 194 μg Fe/g), 158 ± 1 μg Cu/g (certified value, 158 μg Cu/g), and 1.13 ± 0.01% P (certified value, 1.11%). Milk standards ($N = 14$) were determined to contain 1.25 ± 0.01% Ca (certified value, 1.3% Ca), 0.121 ± 0.001% Mg (certified value, 0.120%), and 49.1 ± 1.3 μg Zn/g (certified value, 46.1 μg Zn/g).

The effects of dietary treatments were evaluated by analysis of variance (15). Effects of levels and sources of calcium and their interactions were determined.

RESULTS AND DISCUSSION

The dietary treatments did not affect the body or organ weights of rats. The final average weight of rats was 264 ± 4 g.

Table II. Extent of Utilization of Calcium from Various Supplements as Judged by Tissue Calcium Levels and Apparent Absorption of Calcium

Diet	Kidney Ca (μmol/g)	Tibia Ca (mmol/g)	Apparent absorption of Ca (%) Days 8 & 9	Apparent absorption of Ca (%) Days 31 & 32
4 yogurt	1.40 ± 0.07[a]	4.62 ± 0.10	24 ± 9[a]	18 ± 5
8 yogurt	3.68 ± 1.45	4.75 ± 0.05	11 ± 10	3 ± 5
4 $CaHPO_4$	7.98 ± 2.80	4.85 ± 0.05	33 ± 5	19 ± 18
8 $CaHPO_4$	7.48 ± 4.22	4.90 ± 0.10	22 ± 5	−4 ± 10
4 Ca Mg chelate	1.80 ± 0.32	5.02 ± 0.10	20 ± 8	19 ± 12
8 Ca Mg chelate	3.82 ± 2.05	5.05 ± 0.08	27 ± 7	6 ± 7
4 oyster shell	2.95 ± 1.50	4.78 ± 0.08	20 ± 8	−7 ± 16
8 oyster shell	2.30 ± 0.85	4.80 ± 0.05	19 ± 7	−2 ± 9
Statistical effect of[b]				
Level of Ca	NS	NS	NS	NS
Source of Ca	0.05	0.005	NS	NS
Interaction of level & source	NS	NS	NS	NS

[a] Mean ± SE ($N = 6$). Data expressed as the basis of wet weights of tissues.

[b] Differences expressed as significant ($P < 0.05$ or $P < 0.005$) or not significant (NS).

Table III. Magnesium Utilization by Rats Fed Various Levels and Sources of Calcium

Diet	Kidney (μmol/g)	Tibia (μmol/g)	Apparent absorption (%) Days 8 & 9	Apparent absorption (%) Days 31 & 32
4 yogurt	8.02 ± 0.12[a]	139 ± 2	54 ± 4	52 ± 4
8 yogurt	8.39 ± 0.16	141 ± 1	48 ± 6	47 ± 4
4 $CaHPO_4$	8.64 ± 0.33	139 ± 1	35 ± 15	41 ± 7
8 $CaHPO_4$	8.56 ± 0.25	133 ± 4	24 ± 6	−3 ± 8
4 Ca Mg chelate	8.19 ± 0.12	139 ± 3	41 ± 4	31 ± 4
8 Ca Mg chelate	8.60 ± 0.29	139 ± 2	54 ± 6	36 ± 4
4 oyster shell	8.10 ± 0.16	137 ± 2	56 ± 6	36 ± 14
8 oyster shell	8.23 ± 0.08	138 ± 1	50 ± 5	40 ± 4
Statistical effect of[b]				
Level of Ca	NS	NS	NS	0.05
Source of Ca	NS	NS	0.01	0.005
Interaction of level & source	NS	NS	NS	0.005

[a] Mean ± SE ($N = 6$).
[b] Differences expressed as significant ($P < 0.05$, $P < 0.01$, or $P < 0.005$) or not significant (NS).

Calcium Utilization

Calcium excretion in the feces was significantly increased when rats were fed the 8 mg Ca/g diet rather than the 4 mg Ca/g diet (i.e., 77 vs 34 mg/day on days 31 and 32); this parallels data collected with humans (16,17). However, the percentage apparent absorption of calcium was not statistically affected by either the source or the level of dietary calcium (Table II). Moreover, the level of dietary calcium did not affect tissue calcium levels.

The bioavailability of calcium from the supplements did differ as judged by tibia calcium levels. Tibias of rats fed the chelated calcium and magnesium contained higher levels of calcium than those of rats fed yogurt or oyster shell calcium. This is surprising because, generally, magnesium and calcium are assumed to be antagonistic (18). Among young growing rats the ingestion of additional magnesium in a chelate, a dolomite, or an oyster shell preparation depressed bone calcium levels (8). Perhaps differences in bone turnover due to differences in growth rates or changes in body stores of calcium and magnesium caused the chelated calcium magnesium supplements to be a better source of calcium for the older rats than for the weanling rats.

The source of the calcium also affected kidney calcium levels. Rats fed calcium phosphate dibasic had higher levels of calcium in their kidneys than rats fed other forms of calcium. These older rats seemed somewhat less sensitive to this negative effect of calcium phosphate dibasic than weanling rats (8). This nephrocalcinosis cannot be attributed to magnesium deficiency per se because tissue magnesium levels were in the normal range and were not statistically affected by calcium intake. Other investigators have observed that the addition of magnesium (19), fluoride (19), protein (20), or chloride (21) to diets has lessened nephrocalcinosis induced by an imbalance of dietary calcium, magnesium, and phosphorus.

Magnesium and Phosphorus Utilization

Although the dietary treatments had little effect on

Table IV. Phosphorus Utilization by Rats Fed Various Levels and Sources of Calcium

Diet	Kidney (μmol/g)	Tibia (mmol/g)	Apparent absorption (%) Days 8 & 9	Apparent absorption (%) Days 31 & 32
4 yogurt	91 ± 2[a]	2.90 ± 0.06	75 ± 3	69 ± 3
8 yogurt	92 ± 3	3.03 ± 0.03	63 ± 5	54 ± 2
4 $CaHPO_4$	95 ± 2	3.26 ± 0.06	78 ± 2	73 ± 3
8 $CaHPO_4$	99 ± 2	3.23 ± 0.06	65 ± 3	54 ± 4
4 Ca Mg chelate	88 ± 1	3.19 ± 0.06	61 ± 1	49 ± 6
8 Ca Mg chelate	93 ± 2	2.97 ± 0.06	59 ± 3	40 ± 6
4 oyster shell	90 ± 2	2.77 ± 0.06	76 ± 2	63 ± 6
8 oyster shell	93 ± 2	2.68 ± 0.06	57 ± 2	41 ± 6
Statistical effect of[b]				
Level of Ca	0.05	NS	0.001	0.001
Source of Ca	0.05	0.001	0.05	0.01
Interaction of level & source	NS	NS	NS	NS

[a] Mean ± SE ($N = 6$).
[b] Differences expressed as significant ($P < 0.05$, $P < 0.01$, or $P < 0.001$) or not significant (NS).

Table V. Changes in Gut Functions of Rats Fed Various Levels and Sources of Calcium

Diet	Dry matter digestibility (%) Days 8 & 9	Dry matter digestibility (%) Days 31 & 32	β-Glucuronidase activity (U/mg protein)[b]	pH
4 yogurt	92.9 ± 0.4[a]	93.0 ± 0.4	116 ± 32	7.06 ± 0.05
8 yogurt	91.7 ± 0.2	89.7 ± 1.0	135 ± 28	7.02 ± 0.08
4 $CaHPO_4$	94.2 ± 0.6	93.4 ± 0.3	60 ± 10	7.18 ± 0.05
8 $CaHPO_4$	93.2 ± 0.6	92.1 ± 0.4	68 ± 13	6.60 ± 0.07
4 Ca Mg chelate	91.6 ± 0.6	90.9 ± 0.4	44 ± 30	8.55 ± 0.16
8 Ca Mg chelate	92.0 ± 0.8	89.0 ± 0.6	68 ± 22	9.12 ± 0.09
4 oyster shell	93.8 ± 0.6	92.8 ± 0.5	70 ± 9	7.29 ± 0.18
8 oyster shell	93.0 ± 0.3	91.8 ± 0.6	80 ± 22	7.36 ± 0.22
Statistical effect of[c]				
Level of Ca	NS	0.001	NS	NS
Source of Ca	0.005	0.005	0.05	0.001
Interaction of level & source	NS	NS	NS	0.001

[a] Mean ± SE.
[b] μg of phenalthalein produced/hr/mg of protein.
[c] Differences expressed as significant ($P < 0.05$, $P < 0.001$, or $P < 0.005$) or not significant (NS).

tissue magnesium levels, the dietary treatments did affect the apparent absorption of magnesium (Table III). Initially (days 8 and 9) rats fed either level of calcium phosphate dibasic apparently absorbed magnesium less efficiently than the other rats. At the end of the study (days 31 and 32) rats fed Diet 8 $CaHPO_4$ absorbed magnesium much less efficiently than the other rats. This suggests that eventually rats fed the high levels of calcium phosphate dibasic would have had depressed tissue magnesium levels.

The rats fed the chelated calcium and magnesium, and hence a higher level of magnesium, as well as the rats fed Diet 4 oyster shell tended to absorb magnesium less efficiently than rats fed yogurt. Weanling rats fed magnesium-fortified calcium supplements also absorbed magnesium less efficiently (8).

The level as well as the source of dietary calcium statistically affected the phosphorus content of kidneys. Animals fed the high (8 mg Ca/g diet) level of calcium absorbed phosphorus less efficiently throughout the study but retained more phosphorus in their kidneys than rats fed the moderate (4 mg Ca/g diet) level of calcium (Table IV). Ingestion of additional calcium has been found to depress phosphorus absorption in humans, too (16,17).

Rats fed calcium phosphate dibasic had higher levels of phosphorus in their kidneys and tibias than rats fed other forms of calcium. The ingestion of the chelated calcium and magnesium reduced phosphorus absorption more than the ingestion of the other calcium sources.

General Gut Function and Microflora Metabolism

The digestibility of the total diet was significantly affected by the dietary treatments (Table V). Initially the percentage dry matter digestibility was lower among rats fed the chelated calcium and magnesium or yogurt than among rats fed calcium phosphate dibasic or oyster shells. At the end of the study rats fed the high level of calcium, regardless of the source, had depressed dry matter digestibility. Several investigators have hypothesized that the ingestion of calcium protects animals against colon cancer by forming soaps with

Table VI. Zinc, Copper, and Iron Content of Tissues of Rats Fed Various Levels and Sources of Calcium

Diet	Hematocrit (%)	Liver Fe (μmol/g)	Tibia Fe (μmol/g)	Tibia Zn (μmol/g)	Liver Cu (nmol/g)
4 yogurt	42 ± 1[a]	4.80 ± 0.54	1.81 ± 0.14	2.51 ± 0.05	112 ± 6
8 yogurt	43 ± 1	5.17 ± 0.52	1.77 ± 0.07	2.60 ± 0.05	113 ± 9
4 $CaHPO_4$	44 ± 0	3.71 ± 0.34	2.10 ± 0.16	2.62 ± 0.15	105 ± 6
8 $CaHPO_4$	46 ± 1	5.34 ± 0.52	1.50 ± 0.11	2.54 ± 0.06	98 ± 6
4 Ca Mg chelate	45 ± 1	5.09 ± 0.45	1.68 ± 0.09	2.65 ± 0.06	101 ± 5
8 Ca Mg chelate	44 ± 1	4.33 ± 0.32	1.52 ± 0.07	2.66 ± 0.03	102 ± 3
4 oyster shell	44 ± 1	4.83 ± 0.41	1.83 ± 0.05	2.49 ± 0.06	115 ± 3
8 oyster shell	44 ± 1	4.37 ± 0.32	1.75 ± 0.14	2.52 ± 0.05	105 ± 2
Statistical effect of[b]					
Level of Ca	NS	NS	0.05	NS	NS
Source of Ca	NS	NS	NS	NS	NS
Interaction of level & source	NS	NS	NS	NS	NS

[a] Mean ± SE ($N = 6$). Data expressed on the basis of wet weight of tissues.
[b] Differences expressed as significant ($P < 0.05$) or not significant (NS).

fatty and bile acids in the gut (2). The decreased digestibility of diets when the higher level of calcium was fed suggests that calcium may have chelated potential energy sources, such as fats, and prevented their absorption. The bound compounds should be identified in future work.

We hypothesized that the digestibility of diets would be inversely correlated with β-glucuronidase activity and positively correlated with fecal pH because gut bacteria would have more substrate and produce more acid when the diet digestibility was decreased. However, rats fed the high level of calcium digested dry matter less efficiently but only tended to have a lower specific activity of β-glucuronidase in their feces than rats fed the moderate level of calcium. The elevated fecal β-glucuronidase activity of rats fed yogurt was not due to viable microorganisms in the freeze-dried yogurt (22) but might have been caused by undigested lactose in the guts of rats fed yogurt.

The ingestion of the chelated magnesium and calcium increased the fecal pH more than the ingestion of the other calcium supplements. Perhaps the effect of dietary magnesium on gut microbial activity and colon cancer deserves more study.

Iron, Zinc and Copper Metabolism

Although the calcium intake did not affect the hematocrits or liver iron levels, rats fed the 4 mg Ca/g diet had higher tibia iron levels than rats fed the 8 mg Ca/g diet (Table VI). Moreover, rats fed yogurt absorbed iron significantly less efficiently on days 8 and 9 than rats fed other sources of dietary calcium (data not shown). By days 31 and 32 of the study, the rats had adapted and there was no difference in iron absorption among rats fed the various sources of dietary calcium.

In general the dietary treatments had few effects on zinc and copper metabolism of rats in this study. The apparent absorption of zinc and copper (data not shown) was not affected by either the level or the source of dietary calcium. Thus the ingestion of various calcium sources had more of an effect on iron metabolism than on zinc and copper metabolism; this is consistent with the observations of others (23,24).

ACKNOWLEDGMENTS

This work was supported by the College of Agricultural and Life Sciences, University of Wisconsin—Madison Project 2623, the Wisconsin Milk Marketing Board, the National Institute of Biogerontology, and National Institutes of Health Training Grant 5T32CA09451.

REFERENCES

1. Consensus Development Panel on Osteoporosis. *JAMA* **252**:799–802 (1984).
2. H. Newmark, M. J. Wargovich, and W. R. Bruce. *J. Natl. Cancer Inst.* 72:1323 (1984).
3. G. V. N. Appleton, P. W. Davies, J. B. Bristol, and R. C. N. Williamson. *Br. J. Surg.* 74:523–525 (1987).
4. M. D. Uehling and K. Springen. *Newsweek* Jan. 27:52 (1986).
5. M. J. Nicar and C. Y. C. Pak. *J. Clin. Endocrin. Metab.* **61**:391–393 (1985).
6. M. Goddard, G. Young, and R. Marcus. *Am. J. Clin. Nutr.* **44**:653–658 (1986).
7. M. S. Sheikh, C. A. Santa Ana, M. H. Nicar, L. R. Schiller, and J. S. Fordtran. *N. Engl. J. Med.* 317(9):532–536 (1987).
8. J. L. Greger, C. E. Krzykowski, R. R. Khazen, and C. L. Krashoc. *J. Nutr.* 117:717–724 (1987).
9. American Institute of Nutrition. *J. Nutr.* **107**:1340–1348 (1977).
10. S. Y. Shiau and G. W. Change. *J. Nutr.* **113**:138–144 (1983).
11. O. H. Lowry, J. J. Rosebrough, A. L. Farr, and R. J. Randall. *J. Biol. Chem.* **193**:265–275 (1951).
12. J. L. Greger and S. M. Snedeker. *J. Nutr.* **110**:2243–2253 (1980).
13. C. H. Fiske and Y. Subbarow. *J. Biol. Chem.* **66**:375–400 (1925).
14. Anonymous. In *Food Analysis*, Boehringer, Mannheim, GMBH (1980).
15. T. A. Ryan, B. L. Joiner, and B. F. Ryan. *Minitab Student Handbook*, Duxbury Press, North Scituate, Mass., 1986.
16. J. L. Greger, S. A. Smith, and S. M. Snedeker. *Nutr. Res.* 1:315–325 (1981).
17. H. Spencer, L. Kramer, and D. Osis. *Am. J. Clin. Nutr.* 40:219–225 (1984).
18. M. G. Seelig. *Am. J. Clin. Nutr.* **14**:342–390 (1964).
19. Y. Ericsson, H. Luoma, and O. Ekberg. *J. Nutr.* **116**:1018–1027 (1986).
20. D. E. Yuen and H. H. Draper. *J. Nutr.* **113**:1374–1380 (1983).
21. D. Z. Levine, D. Roy, G. Tolnai, L. Nash, and B. G. Shah. *Am. J. Physiol.* **227**:878–883 (1974).
22. A. Y. Tamime and H. C. Deeth. *J. Food Prot.* **43**:939–977 (1980).
23. J. L. Greger. In C. Kies (ed.), *Nutritional Bioavailability of Iron*, American Chemical Society, Washington, D.C., 1982, pp. 107–120.
24. P. A. Seligman, J. H. Caskey, J. L. Frazier, R. M. Zucker, E. R. Podell, and R. H. Allen. *Obstet. Gynecol.* **61**:356–362 (1983).