Imbalance of Calcium, Magnesium, and Phosphorus in Bone and Other Tissues of Rats Induced by Low Protein and Calcium Deficiency

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Summary Female Wistar rats were separated into 9 groups, and 9 different synthetic diets (each diet contains different level of protein and calcium) were given to each group. After 5 weeks of these dietary regimens, all rats were sacrificed and calcium, magnesium, and phosphorus levels in plasma and various tissues were determined. In calcium-deficient groups, calcium, magnesium, and phosphorus level in bone decreased, plasma calcium level decreased and there was a tendency that magnesium levels in brain and liver and phosphorus level in brain increased. When comparison was made among the calcium-deficient groups, calcium, magnesium, and phosphorus levels in bone were higher in low protein/calcium-deficient group than higher protein/calcium-deficient groups. It is probable that protein deficiency inhibits calcium depletion and consequently the influence of calcium deficiency is less significant in the condition of deficiency in both protein and calcium.

Key Words protein, calcium, magnesium, phosphorus, bone minerals

Calcium is an especially important nutrient for maintenance and growth of bone. However, National Nutritional Survey of Japan revealed that the average calcium intake for Japanese is below the recommended dietary allowance and it is a major nutritional problem that patients with osteoporosis are increasing among the aged people in Japan.

Recently, we conducted nutritional survey studies in aged patients at an institution for aged people and found that the intakes of protein and calcium are insufficient for these people (1). As many reports revealed that dietary protein level influences nutritional status of calcium (2–8) and calcium deficiency induces imbalance of other minerals (9–12), we conducted this study to clarify the effect of dietary protein levels on calcium metabolism in relation to influence on metabolism of other minerals.

MATERIALS AND METHODS

Three-week-old female Wistar rats were separated into 9 groups (①–⑨) of 7–8 rats each and 9 different synthetic diets were given *ad libitum* to these groups. The composition of synthetic diet is shown in Table 1. After 5 weeks of these dietary regimens, rats were anesthetized with sodium pentobarbital and blood was taken from the abdominal aorta until they died by loss of blood. The brain (whole brain), liver, muscle (musculus rectus femoris), and bone (tibia) were then removed. The wet tissue weight was measured. These tissues were wet digested with nitric/perchloric acid mixture in a borosilicate test tube placed in a hot block bath (Model TPB-62, Advantec Toyo Kaisha, Ltd., Tokyo, Japan) at 85°C for 24 h and then heated at 100°C for 5 h. Calcium, magnesium, and phosphorus (total phosphorus) were determined using plasma emission spectrometer ICPS-1000 II (Shimadzu Co., Kyoto, Japan).

The data were expressed as $M \pm SE$. Statistical significance was calculated by Bonferroni's multiple regression analysis. Statistical treatment was performed by SAS's GLM procedure at the data processing center of Kyoto University.

RESULTS

Growth curves are shown in Fig. 1. Growth was retarded significantly ($p < 0.05$) in low protein groups (groups ①, ④, ⑦) as compared to suboptimal protein (15% protein) and optimal protein (20% protein) groups. However, no significant change was observed between suboptimal and optimal protein groups. Difference in calcium intakes did not give a significant change in growth in each protein group.

The calcium concentrations in plasma, brain, liver, and muscles are shown in Table 2. Calcium concentrations in plasma were decreased in calcium-deficient groups (groups ①–③) as compared to control group (group ⑨). In low protein groups (groups ①, ④, ⑦), plasma calcium levels were lower as compared to control group (group ⑨). Calcium level in plasma increased significantly in suboptimal calcium (a half amount of control in the diet) and normal protein group (group ⑥) as compared to control group (group ⑨). In other tissues, changes in calcium concentration showed no marked trend, although calcium concentration of brain was increased significantly in low protein and suboptimal calcium group (group ④) as compared to control group (group ⑨).

Table 3 shows magnesium concentrations in plasma, brain, liver, and muscle. There is a tendency that plasma magnesium concentrations increased in calcium-deficient groups (groups ①–③). However, significant difference was not observed. Brain magnesium concentrations were increased significantly in calcium-deficient low protein and suboptimal protein groups (groups ①, ②) and liver magnesium concentrations increased in calcium-deficient and suboptimal and optimal protein groups (groups ②, ③) as compared to control group (group ⑨).

Table 1. Composition of experimental diet (g/1,000 g diet).

Group	①	②	③	④	⑤	⑥	⑦	⑧	⑨	
Protein	Low	Suboptimal	Deficient	Optimal	Low	Suboptimal	Optimal	Low	Suboptimal	Optimal
Calcium	Deficient	Deficient	Deficient	Suboptimal	Suboptimal	Suboptimal	Optimal	Optimal	Optimal	Optimal
Casein (vitamin free) ¹	50.00	150.00	200.00	50.00	150.00	200.00	50.00	150.00	200.00	200.00
Sucrose	503.00	403.00	353.00	492.65	392.65	342.65	482.30	382.30	332.30	332.30
Starch	300.00	300.00	300.00	300.00	300.00	300.00	300.00	300.00	300.00	300.00
Olive oil	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Cellulose	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Salt mixture	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
(Ca free)										
Vitamin mixture	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Choline chloride	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
CaHPO ₄ ·2H ₂ O	0.00	0.00	0.00	7.10	7.10	7.10	14.20	14.20	14.20	14.20
CaCO ₃	0.00	0.00	0.00	3.25	3.25	3.25	6.50	6.50	6.50	6.50
Salt mixture contained (per 1,000 g diet): NaCl, 1.73 g; Na ₂ HPO ₄ ·H ₂ O, 3.43 g; K ₂ HPO ₄ , 9.45 g; MgSO ₄ , 1.60 g; Fe(C ₆ H ₅ O ₇)·5H ₂ O, 1.15 g; MnSO ₄ , 40 mg; KI, 3 mg; CuSO ₄ ·5H ₂ O, 3 mg; CoCl ₂ ·6H ₂ O, 3 mg; ZnCO ₃ , 3 mg; K ₂ Al ₂ (SO ₄) ₄ ·24H ₂ O, 3 mg; and sucrose to 20 g of salt mixture (Ca free). Vitamin mixture contained (per 1,000 g diet): thiamin hydrochloride, 5 mg; riboflavin, 7.5 mg; niacin, 50 mg; pyridoxine hydrochloride, 5 mg; cyanocobalamin, 50 μg; pantothenic acid, 25 mg; folic acid, 2.5 mg; biotin, 400 μg; ascorbic acid, 187.5 mg; vitamin A palmitate, 62,500 IU; ergocalciferol, 5,000 IU; vitamin E, 5 mg; menadione, 5 mg; inositol, 90 mg; and sucrose to 5 g of vitamin mixture. ¹ Purchased from Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A. The mineral contents in the vitamin-free casein is negligible (24).

Fig. 1. Growth curves.

Table 2. Calcium concentration in plasma, brain, liver, and muscle.

Group	No. of rats	Diet		Plasma ($\mu\text{g/ml}$)	Brain ($\mu\text{g/g}$)	Liver ($\mu\text{g/g}$)	Muscle ($\mu\text{g/g}$)
		Protein (%)	Calcium (mg/kg)				
①	8	5	0	95.5 $\pm$ 2.0*	49.5 $\pm$ 6.1	34.4 $\pm$ 5.8	55.8 $\pm$ 7.8
②	7	15	0	95.2 $\pm$ 4.1*	45.9 $\pm$ 3.3	36.8 $\pm$ 2.2	52.0 $\pm$ 3.6
③	8	20	0	95.2 $\pm$ 4.5*	47.7 $\pm$ 7.6	33.3 $\pm$ 3.2	52.3 $\pm$ 3.8
④	8	5	250	95.4 $\pm$ 5.7*	64.1 $\pm$ 21.7*	33.3 $\pm$ 3.0	52.1 $\pm$ 1.4
⑤	7	15	250	104.8 $\pm$ 2.5	61.1 $\pm$ 28.4	37.7 $\pm$ 2.8	56.7 $\pm$ 8.1
⑥	6	20	250	116.6 $\pm$ 22.8*	63.1 $\pm$ 38.0	36.6 $\pm$ 3.0	51.7 $\pm$ 2.3
⑦	7	5	500	98.1 $\pm$ 2.5*	55.5 $\pm$ 19.4	32.2 $\pm$ 2.4	56.5 $\pm$ 6.9
⑧	6	15	500	105.9 $\pm$ 2.9	52.7 $\pm$ 15.2	34.7 $\pm$ 1.4	53.9 $\pm$ 4.5
⑨	6	20	500	105.5 $\pm$ 6.0	46.7 $\pm$ 7.8	40.1 $\pm$ 2.2	55.9 $\pm$ 2.5

M $\pm$ SD. *Significant difference ($p < 0.05$) as compared to group ⑨ (control group).

Concentrations of phosphorus in plasma, brain, liver, and muscle are shown in Table 4. Phosphorus levels in brain increased in calcium-deficient groups (groups ①–③) and low protein groups (groups ①, ④, ⑦) as compared to control group (group ⑨).

Figures 2–4 show the concentrations of calcium, magnesium, and phosphorus in bone of these individual rats. In bone, calcium levels decreased significantly in calcium-deficient groups (groups ①–③). When comparison was made among the calcium-deficient groups, calcium levels in bone decreased significantly in sub-

Table 3. Magnesium concentration in plasma, brain, liver, and muscle.

Group	No. of rats	Diet		Plasma ($\mu\text{g/ml}$)	Brain ($\mu\text{g/g}$)	Liver ($\mu\text{g/g}$)	Muscle ($\mu\text{g/g}$)
		Protein (%)	Calcium (mg/kg)				
①	8	5	0	13.4 ± 1.5	$160.3 \pm 8.2^*$	238.2 ± 7.4	302.5 ± 7.9
②	7	15	0	14.3 ± 2.2	$158.0 \pm 1.4^*$	$241.5 \pm 7.2^*$	309.0 ± 13.2
③	8	20	0	16.5 ± 1.9	155.9 ± 2.6	$242.4 \pm 5.0^*$	311.5 ± 8.3
④	8	5	250	10.9 ± 2.1	$159.7 \pm 8.5^*$	231.3 ± 6.0	304.5 ± 6.4
⑤	7	15	250	13.6 ± 1.1	153.7 ± 3.4	238.1 ± 5.4	307.3 ± 18.1
⑥	6	20	250	12.4 ± 1.8	153.6 ± 3.0	231.0 ± 3.7	304.5 ± 12.6
⑦	7	5	500	10.5 ± 1.4	154.1 ± 3.5	231.7 ± 8.9	297.8 ± 10.1
⑧	6	15	500	11.6 ± 1.9	152.2 ± 2.0	232.6 ± 10.7	303.8 ± 11.0
⑨	6	20	500	11.4 ± 2.0	152.4 ± 1.8	229.9 ± 1.8	303.6 ± 10.3

M $\pm$ SD. *Significant difference ($p < 0.05$) as compared to group ⑨ (control group).

Table 4. Phosphorus concentration in plasma, brain, liver, and muscle.

Group	No. of rats	Diet		Plasma ($\mu\text{g/ml}$)	Brain ($\mu\text{g/g}$)	Liver ($\mu\text{g/g}$)	Muscle ($\mu\text{g/g}$)
		Protein (%)	Calcium (mg/kg)				
①	8	5	0	119.1 ± 13.7	$3,443 \pm 206^*$	$3,504 \pm 141$	$2,593 \pm 66$
②	7	15	0	133.6 ± 9.1	$3,333 \pm 57^*$	$3,766 \pm 155$	$2,590 \pm 131$
③	8	20	0	130.6 ± 5.4	$3,324 \pm 91^*$	$3,724 \pm 60$	$2,560 \pm 87$
④	8	5	250	128.3 ± 19.5	$3,427 \pm 231^*$	$3,463 \pm 72$	$2,638 \pm 100$
⑤	7	15	250	138.8 ± 11.8	$3,289 \pm 33$	$3,726 \pm 183$	$2,612 \pm 154$
⑥	6	20	250	132.8 ± 8.1	$3,265 \pm 56$	$3,493 \pm 62$	$2,586 \pm 96$
⑦	7	5	500	124.8 ± 12.5	$3,305 \pm 52$	$3,547 \pm 180$	$2,660 \pm 91$
⑧	6	15	500	136.9 ± 9.2	$3,157 \pm 38$	$3,572 \pm 117$	$2,616 \pm 107$
⑨	6	20	500	129.2 ± 8.9	$3,122 \pm 44$	$3,632 \pm 214$	$2,568 \pm 95$

M $\pm$ SD. *Significant difference ($p < 0.05$) as compared to group ⑨ (control group).

optimal and optimal protein groups (groups ②, ③) as compared to low protein calcium deficient group (group ①). Similar relationships were observed also in concentrations of magnesium and phosphorus in bone.

DISCUSSION

Typical changes in minerals of calcium-deficient rats were characterized by hypocalcemia and decrease in calcium levels in bone (10, 11). In addition to these typical changes, the present report revealed that magnesium and phosphorus levels in bone decreased and there were tendencies that magnesium levels in brain and liver and phosphorus level in brain increased. Approximately 99% of calcium, 60%

Fig. 2

Fig. 3

Fig. 2. Calcium concentration in tibia of individual rats. Mean values of groups ①–③ are significantly different ($p < 0.05$) from mean value of group ⑨. Mean values of groups ②, ③ are significantly different ($p < 0.05$) from mean value of group ①.

Fig. 3. Magnesium concentrations in tibia of individual rats. Mean values of groups ①–③ are significantly different ($p < 0.05$) from mean value of group ⑨. Mean values of groups ②, ③ are significantly different ($p < 0.05$) from mean value of group ①.

Fig. 4. Phosphorus concentrations in tibia of individual rats. Mean values of groups ①–③ are significantly different ($p < 0.05$) from mean value of group ⑨. Mean values of groups ②, ③ are significantly different ($p < 0.05$) from mean value of group ①.

of the magnesium, and 80% of phosphorus in the body are in the bones and teeth, and the store of these minerals in these hard tissues is utilized easily in the body (13, 14). It is therefore assumed that these minerals released from the bones to other tissues thereby promoting homeostatic adaptation.

Of note in the present study is the discovery of mineral changes in bone in protein-deficient calcium-deficient rats. Decreased calcium, magnesium, and phosphorus levels in bone in calcium-deficient rats were alleviated significantly when the animals were also deficient in protein. Excessive intakes of protein are known to increase the urinary calcium excretion (3-7) and it can be observed that high protein diet caused a negative net balance of calcium even when calcium intake was sufficient (15-17). Furthermore, it is reported that a diet with excessive meat (18) and calcium deficiency is a factor in causing osteoporosis (19-22). It is probable that substantial body calcium is lost in calcium deficiency when protein intake is high, but calcium equilibrium can be achieved when protein intake is low even if calcium intake is low. The present experiment reconfirmed this fact and in addition, it was clarified that not only calcium but also magnesium and phosphorus could be lost from bones in a similar manner.

Although the exact mechanism of the sparing effect of low protein diet on calcium, magnesium, and phosphorus in bones is unclear, it is assumed that food habits such as consumption of high protein and low calcium could promote the decrease in calcium, magnesium, and phosphorus in bones. Thus, there is a possibility that such an imbalance of calcium and protein intake could have a relation to the development of osteoprotic changes.

Bone and teeth contain certain amounts of magnesium. It may play an important role in the initial formation of apatite and have a significant effect on their physicochemical properties (23). Attention should be paid to magnesium metabolism as well as metabolisms of calcium and phosphorus as the etiology of bone disease.

Another finding of this study is hypocalcemia found in low protein rats. The reason for this finding is not clear yet; protein deficiency may influence intestinal absorption, transport, or homeostatic adaptation mechanisms of calcium.

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