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Original Article

An investigation of excretion of calcium from female mice ingested with boron by using neutron activation analysis

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ABSTRACT

Boron has been considered to play a nutritionally important role in humans and animals, but its biochemical functions are not clearly understood. Though there are signs that boron affects the mineral and hormone metabolisms, there is no comprehensive epidemiological evidence establishing a relationship between a boron intake and osteoporosis due to the excretion of calcium in the bones. In this study, we investigated the influence of boron intake on the calcium excretion of old female mice in the menopause. The concentrations of calcium in backbone, thigh bone, blood, kidney, liver, and spleen were investigated by using instrumental neutron activation analysis.

1. Introduction

Of 116 elements known, only the 19 elements like H, C, N, O, Na, Mg, P, S, Cl, K, Ca, Mn, Fe, Co, Cu, Zn, Se, Mo, and I are considered to be essential in the human diet in common. Some elements like F, Si, V, Cr, Ni, As and Sn have been also suggested to be essential for humans [1]. Though the other elements are considered to be nonessential for humans, several elements may be also essential for specific organisms, not human. For example, boron is required for the growth of certain plants [2], bromine is widely distributed in marine organisms [3], and tungsten is necessary for some microorganisms [4]. Several studies indicated that boron in nutritional amounts may affect the risk of arthritis, osteoporosis, cancer, and the function of the central nervous system [5]. Devirian et al. noted that dietary boron influenced the activity of many metabolic enzymes, as well as the metabolism of steroid hormones and several micronutrients, including calcium, magnesium, and vitamin D [6]. WHO preliminarily concluded that boron is probably essential in human nutrition [7]. The definition of essential elements was determined only from past experiments and can be changed if cases are accumulated by the various experiments currently performed.

Calcium is one of the major elements in higher animals and also essential for life [8]. It is involved in various biochemical functions such as tissues and physiologic processes, including bone and tooth formation, muscle contraction, nerve transmission, and blood clotting, and it serves as a second messenger regulating the actions of many hormones. The recommended dietary allowance for calcium is 1000 mg/day-1200 mg/day for adults [9]. Bone serves as an important storage for calcium, which exits in the form of calcium phosphate. Calcium excretion from the bone is known to be regulated by parathyroid hormone [10–12]. Moreover, dietary boron has been also known to play an important role in helping integrate calcium into bone. It maintains mineral balance for female athletes and sedentary women [13–15], and controls estrogen, and testosterone metabolism in postmenopausal women [13]. The deprivation of dietary boron does not affect on the alteration of magnesium, calcium and phosphorus metabolism by dietary magnesium deprivation in postmenopausal women [16]. Sheng et al. studied the effect of dietary boron supplementation to the estrogen on bone mineral balance in ovariectomized rats [17]. Chapin et al. found that boric acid consumption increased vertebral strength in rats [18]. Boron supplementation in rats and chicks has been shown to increase bone strength [6]. According to the U.S. Department of Agriculture, postmenopausal women with a boron intake of 3 mg a day showed a drop of calcium excretion by 44%, and activation of estrogens and vitamin D, which is needed to absorb calcium [19]. Another experiment

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Table 1
Basal diet (BD) and intake of Ca and B for female mice groups.

Group	Number of mice	Control of diet
Control group	3	BD
Group 1	3	BD + Ca 10 mg/kg/day
Group 2	4	BD + Ca 10 mg/kg/day + B 1 mg/kg/day
Group 3	3	BD + Ca 10 mg/kg/day + B 3 mg/kg/day
Group 4	4	BD + Ca 10 mg/kg/day + B 5 mg/kg/day

showed that boron deprivation could depress the growth of chicks with the effect more marked than when dietary cholecalciferol deficiency. Moreover, the lack of calcification was generally more severe in the boron deprived chicks than cholecalciferol deprived ones [20]. These results suggested that the comprehensive relationship between a boron intake and osteoporosis could be established [21]. The U.S. average dietary intake of boron is 1.5 mg/day [7]. Even though a biochemical function for boron has not been identified, its beneficial actions suggested that an intake of boron over 1 mg/day (but probably not more than 13 mg/day) was desirable [22]. According to the human health risk assessment of boron in drinking water, the Reference Dose was calculated to be 0.3 mg/kg/day, resulting in an acceptable daily boron intake of 18 mg/day [23].

In this study, we investigated the effect of calcium excretion with boron intake in several organs such as backbone, thigh bone, blood, kidney, liver, and spleen in postmenopausal female mice by using instrumental neutron activation analysis [24,25].

2. Experimental

2.1. Sampling and sample preparation

11-week-old female BALB/C mice, weighing about 30 g, were purchased from Saemdaco (Osan, Gyunggi-do, Korea) and were housed in stainless steel cages in an environmentally normal animal room at 22 ± 2 °C with a relative humidity 55 ± 5%, a normal 12-h light and dark cycle, and food and water ad libidum. As shown in Table 1, each group was controlled to be fed with limited diets for four weeks. The average intake of calcium by the basal diet was estimated as about 25 mg/day, and the boron intake was negligible. Basal diet, which was produced for rat and mice feed, is composed of corn, wheat bran, steam fish meal, soybean meal, limestone, salt, vitamin, and mineral. Its detail ingredient is listed in Table 2. Calcium concentration is about 7.90 g/kg but boron is excluded in basal diet. The deionized water were supplied in plastic cups.

BALB/C mice were randomly assigned to five groups of five and given the calcium an boron solution via the oral route: (1) " control group " were given physiological saline; (2) calcium solution 10 mg/kg; (3) calcium solution (10 mg/kg/day) + boron solution (1 mg/kg/day); (4) calcium solution (10 mg/kg/day) + boron solution (3 mg/kg/day); (5) calcium solution (10 mg/kg/day) + boron solution (5 mg/kg/day). Calcium and boron solution were administered orally to mice with oral syringe once a day for 120 consecutive days. Each mouse was weighed weekly. Any humane handlings without feeding were restricted to avoid interferences for the behavioral test as soon as possible. On day 120, each group of mice was anesthetized in an ether-saturated chamber and experimental samples including backbone, thigh bone, blood, kidney, liver and spleen were taken for the analysis. All experiments were conducted in accordance with the guide for the care and use of laboratory animals [26].

2.2. Neutron activation analysis

The prepared samples were irradiated in the NAA#1 and NAA#2 of the HANARO research reactor in Korea Atomic Energy Research Institute. The thermal neutron flux of NAA#1 and NAA#2 was 3.8×10^{13} n/cm^{2}s and 2.7×10^{13} n/cm^{2}s, respectively. For the analyses of calcium, the samples were irradiated at the NAA#1 irradiation hole. An Al-0.1% Au wire and a pure Fe wire were used for the monitoring of neutron fluence during the irradiation for short-lived and long-lived nuclides, respectively.

Table2
A formula of basal diet (BD) and Its ingredients.

Formula amount			Vitamine		
Ingredient	Unit	Value	Ingredient	Unit	Value
Animal Product	%	3.4	A	IU/kg	7357.00
Cereal	%	91.0	B1	mg/kg	4.33
Vitamin	%	0.1	B3	mg/kg	3.65
Mineral	%	0.1	B6	mg/kg	4.27
Other	%	5.4	B12	mg/kg	15.00
Total	%	100	D3	IU/kg	1002.52
Chemical composition			E	mg/kg	42.43
Moisture	%	12.5	K3	mg/kg	1.05
Crude protein	%	23.5	Niacin	mg/kg	27.20
Crude Fat	%	5.9	Folic Acid	mg/kg	1.39
Crude Ash	%	5.9	Inositol	mg/kg	2.07
Crude Fiber	%	3.9	Biotin	mg/kg	1.49
NFE	%	48.3			
ME	kcal/g	3.32			
Amino Acid			**Mineral**		
Lysine	%	1.29	Fe	mg/kg	119.67
Methionine	%	0.42	Zn	mg/kg	71.94
Cystein	%	0.37	Mn	mg/kg	21.57
Tryptophan	%	0.29	Cu	mg/kg	11.12
Arginine	%	1.48	I	mg/kg	1.28
Histidine	%	0.62	Se	mg/kg	1.46
Threonine	%	0.89	Co	mg/kg	0.0051
Isoleucine	%	0.97	S	g/kg	2.39
Valine	%	1.11	K	g/kg	8.79
Leucine	%	1.99	Ca	g/kg	7.90
Phenylalanine	%	1.14	P	g/kg	6.98
Tyrosine	%	0.85	Na	g/kg	2.31

Table 3
Results of quality control analyses of NIST SRM 1400 bone ash and NIST SRM 1572b bovine liver.

Element	NIST SRM 1400 bone ash				NIST SRM 1577b bovine liver			
	INAA [mg kg^{-1}]	RSD [%]	Certified [mg kg^{-1}]	Coincidence Factor [%]	INAA [mg kg^{-1}]	RSD [%]	Certified [mg kg^{-1}]	Coincidence Factor [%]
Ca	37.43 ± 0.86	2.3	38.18 ± 0.13	98	126 ± 3	2.1	131 ± 10	96
Mg	0.652 ± 0.03	4.2	0.684 ± 0.013	95	635 ± 10	1.5	620 ± 42	102
Mn				103	10.1 ± 0.2	2.3	10.46 ± 0.47	97
Fe	667 ± 29	4.3	660 ± 27	101	195 ± 6	3.2	197 ± 0.65	99
Sr	240 ± 7	2.8	249 ± 7	96	94.2 ± 3.4	3.6	95.3 ± 4.2	99
Zn	181 ± 3	1.9	181 ± 3	100	181 ± 4	2.2	181 ± 1.0	100
Mo				104	3.25 ± 0.80	2.4	3.30 ± 0.13	99

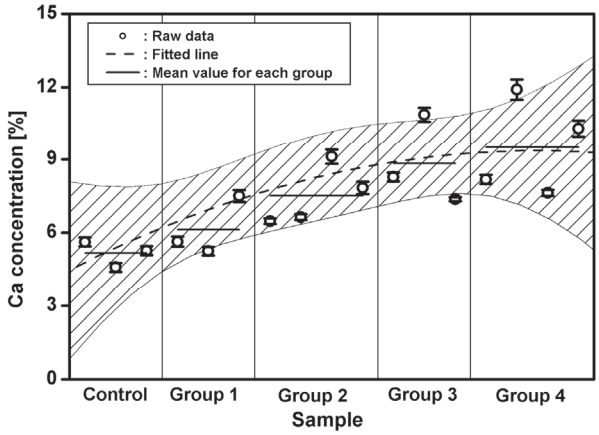

Fig. 1. Variation of Ca concentration in a backbone.

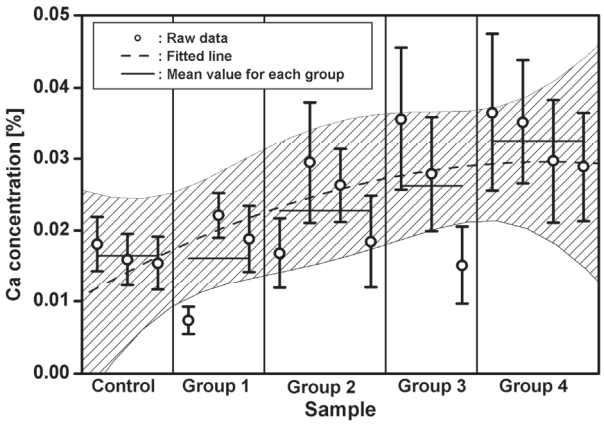

Fig. 3. Variation of Ca concentration in blood.

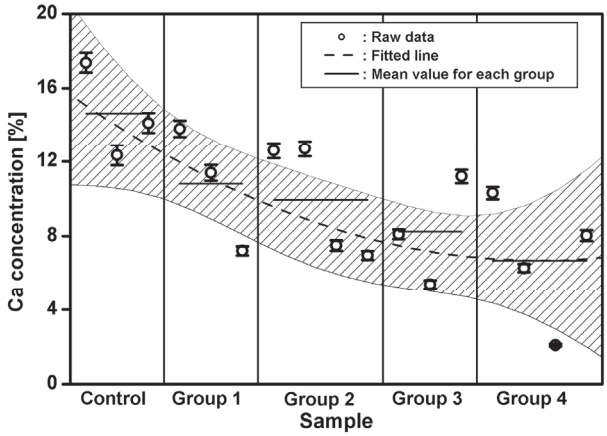

Fig. 2. Variation of Ca concentration in a thigh bone.

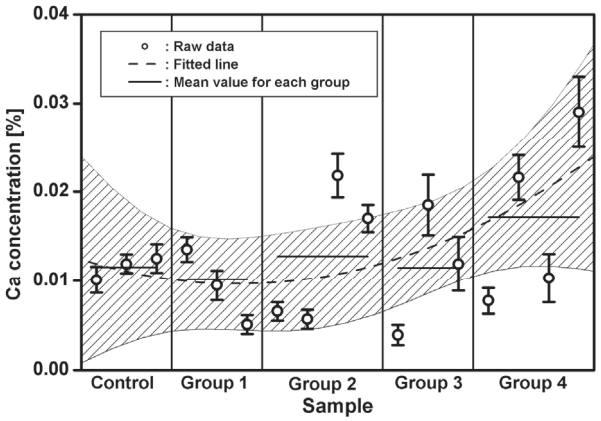

Fig. 4. Variation of Ca concentration in a kidney.

The certified reference materials NIST SRM 1400 bone ash [27] and NIST SRM 1572b bovine liver [28] were utilized for quality control in the basal diet and supplementary diet as shown in Table 3, which shows the relative standard deviation (RSD) in the measurement and coincidence factor between the measured and certified concentrations. Gamma-ray spectra from the irradiated samples were analyzed with the HYPERGAM [29] based on HYPERMET gamma-ray analysis routine, and the newly developed KAERI-NAA software was used for the determination of the elemental concentrations in the samples. The statistical analysis tools in the ORIGIN ver. 8.0 was used. The statistical data like confidence band were generated to examine the differences and trend of the elemental concentration of calcium in the each organ.

3. Results and discussion

As shown in Fig. 1 and Fig. 2, boron supplement influenced the concentrations of calcium in the backbones and thigh bones. The concentrations were given in weight percent. The hatched region was within lower and upper 95% confidence limits of the fitting of the measured data. The concentrations for the control group were about 5.2% and 14.6% for backbone and thigh bones, respectively. The value in the backbone increased by 4% after intake of calcium supplement and continued to increase by two times that for the control group when the intake was increased to 5 mg/kg day. On the other hand, the concentration of calcium in thigh bones decreased by 7% for the group 4 according to the boron intake.

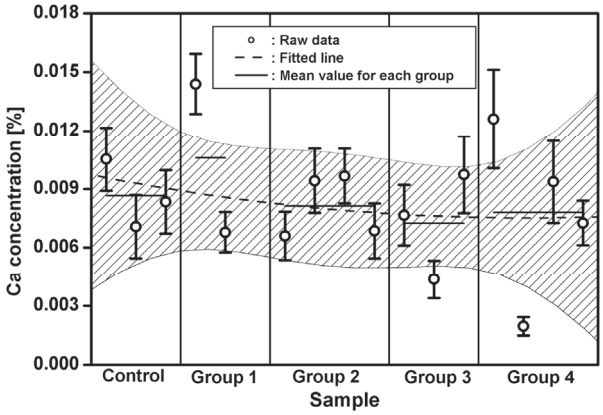

Fig. 5. Variation of Ca concentration in a liver.

These conflicting trends for different bones suggested that the biochemical function of boron in animals must be carefully dealt with, especially for the mice in the menopause.

The concentrations of calcium in other organs including blood showed different trends. As shown in Fig. 3, the value in the blood gradually increased by three times that for the control group. The normal concentration of boron in blood is apparently between 0.1 and 0.2 μg/ml [30]. Fig. 4 shows that the value in the kidney was slightly varied from about 0.011% to about 0.017%, but those in the liver and spleen decreased according to boron intake as shown in Figs. 5 and 6, respectively. The trends in the backbone and thigh bone were due to the limited sample of the old female mice in the menopause and may not reflect the trend of the concentrations of calcium similar to that of the bones.

These results may not give comprehensive epidemiological evidence establishing a clear relationship between boron intake and osteoporosis, which is due to the excretion of calcium in the bones. These can be examples and clues which show that boron repletion besides boron depletion in animals may lead to the enhancement of excretion of calcium in the bones, especially of at least old female mice in the menopause.

4. Conclusions

Influence of boron intake on the calcium excretion of old female mice in the menopause was investigated. The concentration of calcium in backbone increased according to the boron intake, while that in the thigh bone decreased. Those in other organs also show different trends for the mice in the menopause. The calcium level in the liver and spleen tended to decrease by less than 0.005%. The calcium excreted from the organs increased the level in the blood and the kidney. The complexity of calcium excretion from the bone and organs means that the relationship between the intake of elemental boron as a dietary supplement and the calcium integration in the bone is needed to be more investigated, especially for the menopausal woman. For a clear understanding of the biochemical function of boron repletion or depletion in animals, further supplementary experiments on the biochemical function of boron influencing the calcium excretion would be done for the mice in various conditions of boron and calcium intake.

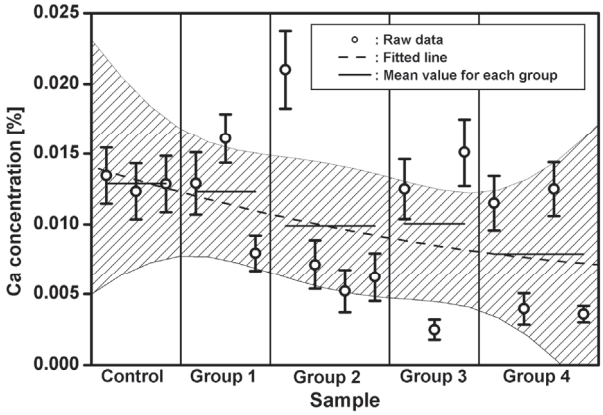

Fig. 6. Variation of Ca concentration in a spleen.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.net.2020.04.010.

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