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Zinc deficiency negatively affects alkaline phosphatase and the concentration of Ca, Mg and P in rats*

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Abstract

Zn is an essential nutrient that is required in humans and animals for many physiological functions, including immune and antioxidant function, growth, and reproduction. The present study evaluated whether Zn deficiency would negatively affect bone-related enzyme, ALP, and other bone-related minerals (Ca, P and Mg) in rats. Thirty Sprague Dawley rats were assigned to one of the three different Zn dietary groups, such as Zn adequate (ZA, 35 mg/kg), pair fed (PF, 35 mg/kg), Zn deficient (ZD, 1 mg/kg) diet, and fed for 10 weeks. Food intake and body weight were measured daily and weekly, respectively. ALP was measured by spectrophotometry and mineral contents were measured by inductively coupled plasma- mass spectrophotometer (ICP-MS). Zn deficient rats showed decreased food intake and body weight compared with Zn adequate rats ($p<0.05$). Zn deficiency reduced ALP activity in blood (RBC, plasma) and the tissues (liver, kidney and small intestine) ($p<0.05$). Also, Zn deficiency reduced mineral concentrations in rat tissues (Ca for muscle and liver, and Mg for muscle and liver) ($p<0.05$). The study results imply the requirement of proper Zn nurture for maintaining bone growth and formation.

Key Words: Zinc, alkaline phosphatase, bone-related minerals (Ca, P, Mg), rats

Introduction

The major Zn deficiency in experimental animals and to lesser extent in human is associated with skeletal and growth retardation due to anorexia, weight loss and poor food efficiency (Vallee & Falchuk, 1993). Rats fed with a Zn-deficient diet consume less food and therefore have significantly reduced growth which has been associated with abnormalities in bone growth, bone formation and mineralization (Roth, 2003; Yamaguchi & Yamaguchi, 1986). In addition, Zn deficiency weakens protein synthesis (Giugiano & Millward, 1987), making Zn an essential factor for the synthesis of proteins (Hendy *et al.,* 2001). Also, Zn is known to be necessary for normal collagen synthesis and mineralization of bone that has been demonstrated to be essential for normal growth of both human and animal skeletal systems (Bougle *et al.,* 2004; Doherty *et al.,* 2002; Eberle *et al.,* 1999; Elmstachl *et al.,* 1998; Hosea *et al.,* 2004; Hyun *et al.,* 2004; Ovesen *et al.,* 2001; Peretz *et al.,* 2001; Rossi *et al.,* 2001; Seco *et al.,* 1998; Suwarnasarn *et al.,* 1982;). It therefore has been considered that Zn can be a critical factor to affect skeletal development and bone formation. However, it has not been clearly clarified how Zn controls bone formation, yet.

Bone formation is regulated through bone remodeling (Karsdal *et al.,* 2007). Bone remodeling is comprised of two well defined cellular events that occur sequentially. There is first the resorption of preexisting bone by the osteoclasts then *de novo* bone formation by the osteoblasts (Ducy *et al*., 2000; Frost, 1969). Osteoporosis, a bone remodeling disease, is the most common bone disease (Sambrook & Cooper, 2006) and a lot of efforts are being made to clarify that particular disease at menopause. The coordination of bone remodeling to maintain bone mass can be affected by Zn. Zn stimulates bone metabolism in rats and bone formation in tissue cultures by increasing bone DNA and protein synthesis (Yamaguchi & Yamaguchi, 1986; Yamaguchi *et al.,* 1988). In addition, Zn was shown to exert an inhibitory effect on the activity of the osteoclasts responsible for bone resorption *in vitro* (Holloway *et al.,* 1996; Moonga & Dempster, 1995). In the recent study, overexpression of the ZIP1, ubiquitous

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zinc transporter in the cells, induces an osteogenic phenotype in human mesenchymal stem cells (MSCs) through increasing osteoblast differentiation genes, including bone-specific zinc finger transcription factor Cbfa1/Runx2 and alkaline phosphatase (ALP) (Tang *et al*., 2006). This report investigated osteogenic Zn function on the bone formation at cellular and molecular level.

Alkaline phosphatase (ALP) is an enzyme which is synthesized by the liver, bone, and less amount by intestines and kidney. As the name implies, this enzyme works best at an alkaline pH (pH ≥10), and thus the enzyme itself is inactive in the blood. ALP removes phosphate group (known as dephosphorylation) from many types of molecules, including nucleotides, proteins, and alkaloids. For Ca crystallization on bone formation, Ca can be crystallized in an alkaline environment, but dissolved in an acidic state (Shinozaki & Pritzker, 1996). Alkaline phosphatase is synthesized and secreted by osteoblasts during the maturation of osteoblast phenotypic period, three consecutive phases of proliferation, extracellular matrix maturation and mineralization (Stein & Lian, 1993), in bone tissue. Thus it makes sense that osteoblasts synthesize and secrete alkaline phosphates creating a local bone environment of alkalinity by splitting off phosphorus (an acidic mineral) creating an alkaline pH to help build bone Ca mineralization. ALP is normally presents in high concentrations in growing bones and it is also known as Zn-dependent enzyme.

The present study investigated the effects of Zn deficiency on ALP, which stimulates extracellular matrix Ca mineralization and minerals (Ca, Mg and P), which are the ECM components of Ca mineralization in rats. To avoid the effect of the decreased food intake which is the major sign of Zn deficiency, we also designed the experiment to pair-fed the rats the normal Zn-adequate diet with the decreased amount of Zn-deficient dietary group. Again, the study aims to elucidate the ALP and trace minerals (Ca, Mg and P) in blood and various tissues of Zn-deficient rats.

Materials and Methods

Animals and diets

Thirty Sprague Dawley rats (100 g, 4 weeks of age) were obtained from SLC Inc, (Shizuoka, Japan), and were individually housed in stainless steel wire-bottom cages in an environmentally temperature controlled room at 22 ± 0.5℃ with an alternate 12 h light and dark cycle. The rats were grouped into one of the three Zn dietary groups (Zn-adequate, ZA; pair-fed, PF; and Zn-deficient group, ZD. n=10 per group) and were given a modified egg albumin-based AIN-76 diet that contained modified mineral mixture (Harlan Tekland, Wisconsin USA) provided with 35 and 1 mg Zn/kg diet for ZA and ZD diets, respectively, as previously described (Kwun *et al.*, 2007; Lee *et al.*, 2003). To control for reductions in food intake that characteristically occur in ZD rats, a PF group was fed the same amount of ZA food eaten by ZD rats. All rats had free access to distilled and deionized water from plastic bottles with silicon stoppers. ZA and ZD rats were given free access to food.

The diet was stored at 4℃ in plastic containers and handled with plastic gloves and appropriate utensils to avoid Zn contamination. Food intake was recorded daily and body weight was recorded every week. The care and use of the rats strictly followed "The Guiding Principles for the Care and Use of Animals" (National Research Council, 1996) and the ANU institutional guidelines. To ensure that the rats would be given appropriate amounts of Zn, the diets were analyzed using inductively coupled plasma spectrophotometer (ICP; Flame Modula S, Germany). Samples were digested using nitric acid (Fluka, Switzerland) and diluted with trace element free 0.125 M HCl (Fluka, Switzerland) at 1:10. Analyzed Zn concentration of the diets showed that the nominal Zn concentration agreed with the actual Zn concentration having 0.3 ± 0.1 mg/kg feed for Zn-deficient and 35.1 ± 12.9 mg/kg feed for both ZA and PF diets. Zn deficiency took place with a Zn intake of <1 mg Zn/kg (Chesters & Quarterman, 1970; Scrimgeour *et al.,* 2007). The analytical accuracy of the method for mineral analysis was tested using a standard reference material (SRM) obtained from National Institute of Standards and Technology (NIST SRM 1577b, bovine liver, USA) and the recovery of Zn was 97.3 ± 8.0%.

Blood and tissue collection

On the last day of dietary Zn treatment for ZA and ZD groups, and one day later for the PF group, the assigned rats were anesthetized with ketamin/HCl (10 mg/kg body weight). Blood sample was obtained from the abdominal aorta using a heparinised syringe and centrifuged at 10,000 × g for 15 min to obtain plasma and red blood cells. The tissues [liver, muscle, kidney, small intestine (SI) and white adipose tissue (WAT)] were collected for ALP, Zn and minerals (Ca, Mg, and P) determination. The tissue and plasma samples were then snap-frozen in liquid nitrogen and stored at -80℃.

Tissue and blood alkaline phosphatase (ALP) activity assay

Tissues (liver, muscle, kidney, SI) and blood (plasma and red blood cell) alkaline phosphatase were measured by enzymatic activity. Tissues and RBCs were washed with PBS and homogenized in 1 mL of 0.25 M sucrose (Sigma, USA) on ice. The cytosol was centrifuged for 15 min at 12,000 × g. The supernatant was kept at -20℃ until analysis. The ALP activity in cytosol of tissues, RBCs and plasma was measured by using *p*-nitrophenyl phosphate as the substrate. In brief, aliquots of tissue homogenates and RBCs and plasma were mixed with assay buffer containing 10 mM p-nitrophenyl phosphate in 0.1 M sodium carbonate buffer, pH 10, supplemented with 1 mM $MgCl_2$

Table 1. Daily food intake and body weight of the rats during the whole experimental period (42 d) by dietary zinc level[1]

Day	Food intake			Body weight		
	ZA	PF	ZD	ZA	PF	ZD
0	14.5 ± 0.2	14.9 ± 0.3	14.4 ± 0.6	120.1 ± 2.6	120.5 ± 2.4	120.6 ± 2.4
7	14.8 ± 0.3	15.3 ± 0.4	15.3 ± 0.5	152.2 ± 1.9	143.8 ± 2.8	150.1 ± 2.5
14	17.0 ± 0.3^{a}	13.9 ± 0.6^{b}	11.0 ± 0.7^{c}	204.4 ± 2.7	197.1 ± 2.9	200.2 ± 3.4
21	18.9 ± 0.4^{a}	13.2 ± 1.4^{b}	10.3 ± 1.3^{b}	253.0 ± 3.1^{a}	210.3 ± 2.8^{b}	207.9 ± 3.0^{b}
28	19.8 ± 0.6^{a}	11.0 ± 1.5^{b}	12.3 ± 1.7^{b}	298.3 ± 4.2^{a}	220.7 ± 2.0^{b}	211.7 ± 2.7^{b}
35	19.0 ± 0.9^{a}	10.4 ± 0.9^{b}	11.4 ± 1.2^{b}	328.8 ± 4.6^{a}	227.8 ± 2.8^{b}	216.1 ± 1.7^{c}
42	18.3 ± 0.4^{a}	10.1 ± 1.7^{b}	9.5 ± 0.9^{b}	347.8 ± 5.2^{a}	225.0 ± 3.9^{b}	219.7 ± 2.5^{b}

[1] Values are mean ± SE (n=10 per group). ZA (Zn-adequate, 35 mg Zn/kg diet), PF (pair-fed, 35 mg Zn/kg diet) and ZD (Zn-deficient, 1 mg Zn/kg diet). Daily food intake and weekly body weight were measured. Different superscripts mean significantly different among Zn dietary group at $p<0.05$ by Tukey, one-way ANOVA within each food intake or body weight.

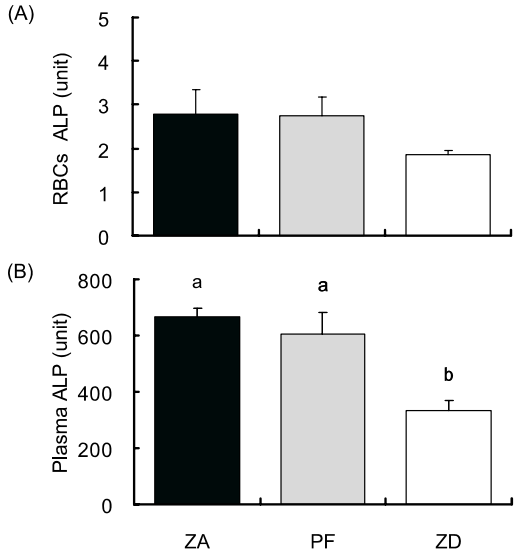

Fig. 1. Activity of alkaline phosphatase (ALP) in red blood cell (RBCs, A) and plasma (B) of Zn-adequate (ZA), pair-fed (PF) and Zn-deficient (ZD) rats. Values are mean ± SE (n=7 per for RBCs; n=7 for ZA and PF, n=5 for ZD for plasma). Different superscripts mean significantly different among Zn dietary group at p <0.05 by Tukey, one-way ANOVA. One unit of ALP activity is expressed as the amount of *p*-nitrophenol phosphate as products being converted from *p*-nitrophenyl phosphate as substrate for 30 minute reaction time (RBCs ALP 1 unit = 1 nmole *p*-nitrophenol phosphate/30 min/mg protein ; plasma ALP 1 unit = 1 nmole *p*-nitrophenol phosphate/30 min/ml)

and followed by an incubation at 37℃ for 30 min. After adding 1 M NaOH, the amounts of *p*-nitrophenol liberated in the assay mixtures were measured using a spectrophotometer at 420 nm. (unit; mmoles/min/mg protein or mU/mg protein) (Bessey *et al*., 1946). Plasma ALP activity was expressed as *p*-nitrophenol phosphate nmole /ml/30 mins as 1 unit. Protein concentration of tissues and RBCs was estimated by the method of Lowry *et al.* (1951) with bovine serum albumin (BSA) as the substandard.

Tissue Zn and other mineral (Ca, Mg and P) analysis

Tissues (liver, muscle, kidney, SI and WAT) in trace element free 0.125 M HCl were homogenized before trace mineral assay. Tissues were dried and ashed at 500℃ in furnace for overnight. Tissues were wet digested using concentrated nitric acid and diluted with trace element free 0.125 M HCl at 1:2 to 1:30 as appropriate. The diluted samples were filtered using 0.2 μm syringe filter (Corning, USA) and measured using inductively coupled plasma spectrophotometer (ICP; Flame Modula S, Germany) for Zn, Ca, Mg and P. Protein concentration in tissues was analyzed by Lowry method.

Statistical analysis

The data were expressed as means ± SE and analyzed using SPSS program. Differences were considered significant at $p<0.05$. Statistical analysis of the data was performed by one-way ANOVA followed by Tukey *post hoc* test to test the significance in the effect of different Zn dietary groups.

Results

Body weight and food intake

Daily food intake and weekly body weight during the whole experimental period are shown in Table 1. Rats fed ZD diets consumed significantly less food than rats fed ZA diets since 2nd week of the experimental diet feeding ($p<0.05$). Also, rat consuming the ZD diets exhibited significantly lower body weight compared to PF or ZA ($p<0.05$). Mean body weight at the final 7th week, ZA group (347.8 g) showed about 100 g higher body weight than that of PF (225.0 g) or ZD group (219.7 g).

Tissue and blood alkaline phosphatase (ALP) activity

Alkaline phosphatase (ALP) activities in blood subfractions, as plasma and red blood cells (RBCs) during different dietary Zn periods (ZA, PF and ZD) are shown in Fig. 1. ALP activity in the plasma of the ZD rats was significantly decreased compared with the ZA rats ($p<0.05$). Also ALP activity in the RBCs showed the same pattern of plasma ALP activity even without significance.

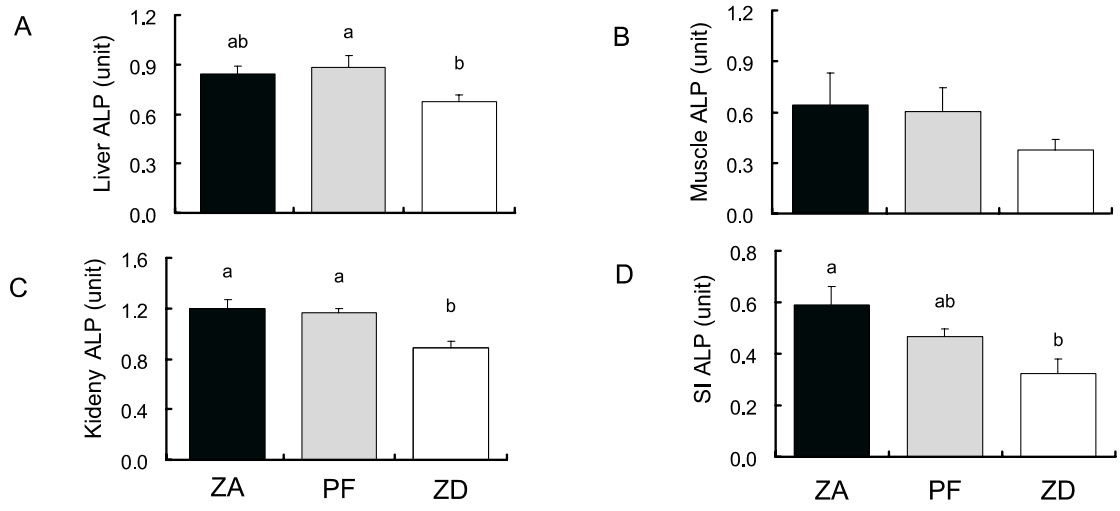

Fig. 2. Activity of alkaline phosphatase (ALP) in liver (A), muscle (B), kidney (C) and SI (small intestine, D) of Zn-adequate (ZA), pair-fed (PF) and Zn-deficient (ZD) rats. Values are mean ± SE (n=8-10 per group). Different superscripts mean significantly different among Zn dietary group at $p<0.05$ by Tukey, one-way ANOVA. Tissue ALP 1 unit = 1 nmole p-nitrophenol phosphate/30 min/mg protein).

Table 2. Bone-related mineral (Ca, Mg and P) concentration in rat tissues (liver, muscle, kidney, small intestine and white adipose tissue) fed different dietary zinc level[1]

	Ca concentration (μg Ca/g dry tissue)			P concentration (mg P/g dry tissue)			Mg concentration (μg Mg/g dry tissue)		
Tissues	ZA	PF	ZD	ZA	PF	ZD	ZA	PF	ZD
Liver	121.9 ± 5.0^{a}	49.1 ± 6.8^{b}	68.0 ± 6.4^{b}	2.24 ± 0.17	2.57 ± 0.07	2.10 ± 0.20	153.0 ± 18.8	156.5 ± 16.8	112.1 ± 0.4
Muscle	61.7 ± 0.8^{a}	41.6 ± 3.0^{b}	44.4 ± 2.8^{b}	1.30 ± 0.52	1.26 ± 0.05	1.54 ± 0.14	188.3 ± 0.3^{a}	57.3 ± 9.4^{b}	48.5 ± 14.7^{b}
Kidney	122.0 ± 12.0^{a}	98.9 ± 15.3^{b}	104.5 ± 2.2^{b}	2.15 ± 0.15	2.15 ± 0.28	2.25 ± 0.05	191.6 ± 17.4	184.6 ± 19.9	186.0 ± 4.2
Small intestine	132.7 ± 16.2^{a}	94.2 ± 7.6^{ab}	83.3 ± 8.6^{b}	1.65 ± 0.20	1.38 ± 0.11	1.30 ± 0.13	140.2 ± 9.1^{a}	105.2 ± 8.1^{ab}	87.3 ± 11.6^{b}
White adipose tissue	27.7 ± 3.2^{b}	44.8 ± 4.2^{a}	25.2 ± 2.3^{b}	0.18 ± 0.03^{b}	0.54 ± 0.11^{a}	0.22 ± 0.04^{b}	25.3 ± 4.7^{b}	42.5 ± 6.5^{a}	22.5 ± 5.3^{b}

[1] Values are means ± SE (n=3-4 per group). ZA (Zn-adequate, 35 mg Zn/kg diet), PF (pair-fed, 35 mg Zn/kg diet) and ZD (Zn-deficient, 1 mg Zn/kg diet). Different superscripts mean significantly different among Zn dietary group at $p<0.05$ by Tukey, one-way ANOVA.

ALP activity in tissues, as liver, muscle, kidney and SI by different dietary Zn groups (ZA, PF and ZD) are shown in Fig. 2. ALP activity in liver, kidney and SI of the ZD rats was significantly lower than that of ZA or PF rats ($p<0.05$), however ALP activity in tissues, as liver, kidney and SI between ZA and PF was not different. ALP activity in the muscle showed the same pattern without significance. In this study, ALP activities both in tissues (liver, kidney and SI) and plasma of ZD rats were decreased compared with the ZA rats.

Tissue Zn concentration

Zn concentrations in tissues, as liver, muscle, kidney, SI and WAT by different dietary Zn levels (ZA, PF and ZD) are shown in Fig. 3. Dietary Zn did not affect the Zn concentration in tissues such as liver, muscle and kidney. Zn concentration in SI of ZD rats was decreased compare to ZA or PF controls ($p<0.05$). Unexpectedly, Zn concentration in WAT of PF rats was higher than that of ZA or ZD ($p<0.05$).

Tissue mineral (Ca, P and Mg) concentration

Ca concentration in tissues, as liver, muscle, kidney, SI, and WAT by different dietary Zn levels (ZA, PF and ZD) are shown in Table 2. Ca concentration in tissues showed consistent pattern depending on different dietary Zn periods. Ca concentrations in liver, muscle and SI of ZD rats were significantly lower than those in ZA rats ($p<0.05$). However Ca concentration in WAT of PF rats was increased compared with ZA and ZD ($p<0.05$), as the pattern was the same for WAT Zn concentration. P concentration in tissues did not differ by dietary Zn groups among ZA, PF and ZD rats, except WA tissue. Mg concentrations were decreased in muscle and SI ($p<0.05$) of ZD group. Zn and all other minerals (Ca, P and Mg) were increased in PF group compared with ZA or ZD. In tissues, ZD rats had lower mineral concentrations (Zn, Ca, Mg and P) than ZA rats, respectively. However, Zn, Ca, Mg and P concentrations in WAT of PF rats were significantly increased compared with ZA and ZD ($p<0.05$). It suggests that further studies on how dietary Zn level affects WAT in rats are needed.

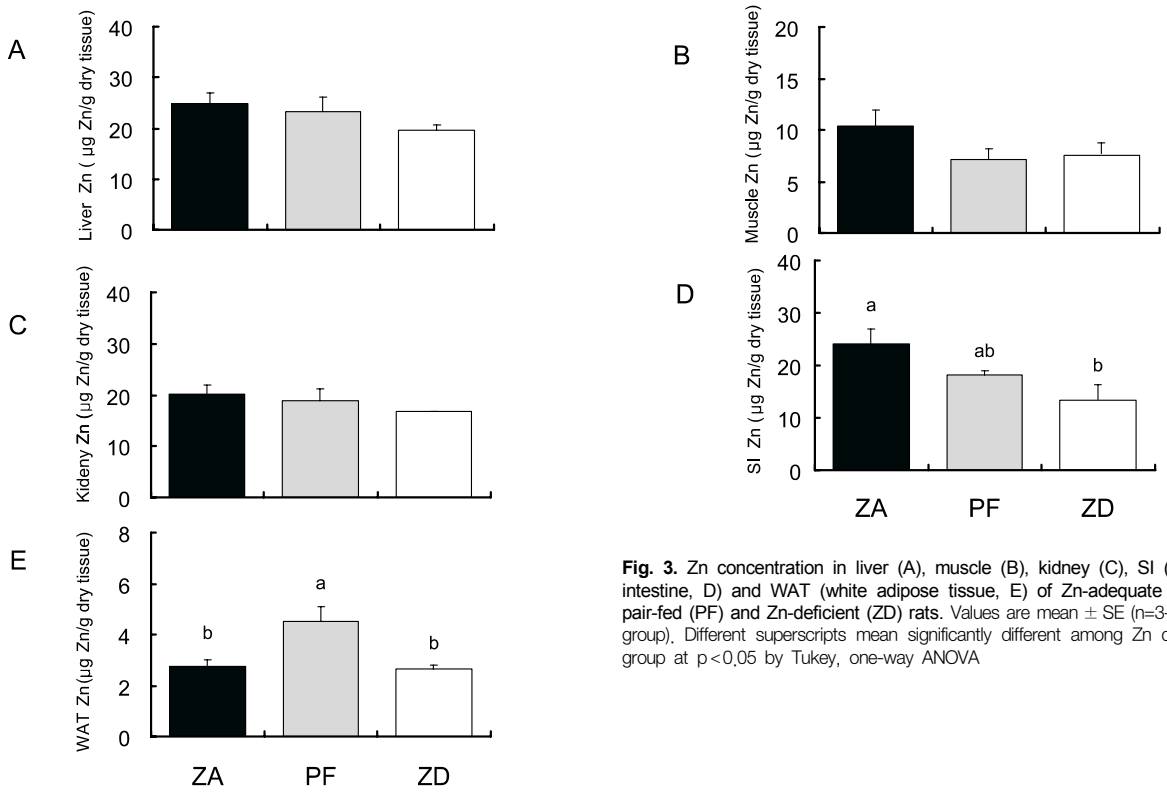

Fig. 3. Zn concentration in liver (A), muscle (B), kidney (C), SI (small intestine, D) and WAT (white adipose tissue, E) of Zn-adequate (ZA), pair-fed (PF) and Zn-deficient (ZD) rats. Values are mean ± SE (n=3-4 per group). Different superscripts mean significantly different among Zn dietary group at $p < 0.05$ by Tukey, one-way ANOVA

Discussion

The study aimed that Zn deficiency decreases ALP and bone-related major minerals (Ca, Mg and P) in rats. Thus, the present study investigated the effects of dietary Zn deficiency on food intake, body weight following the patterns of bone formation enzyme (ALP) and bone related minerals (Ca, Mg, and P) in rats on the 6-week dietary Zn treatment. Many previous studies of Zn deficiency in rats have indicated that severely Zn deficient diets cause a decrease in body weight and bone growth due in part to decreased appetite and impaired protein synthesis (Eberle *et al.,* 1999; Hosea *et al.,* 2004; Rossi *et al.,* 2001; Underwood, 1981). In the study conducted, generally, rats from all the feeding groups increased their body weights within the 6-week feeding period. However, ZA group significantly increased the body weight including food intake as compared to the PF and Zn-deficient groups which strongly confirms previous claims that Zn deficiency slows down appetite regulation. Interestingly, food intake was not affected until seven days later which resulted from the casein-based diet as protein source until day 12. After then, the protein source was changed to egg white, which contains naturally less Zn compared to casein, to induce much severe Zn deficiency. Therefore, there might be a regulatory impulse generated and that Zn deficiency might have reached the critical level that induced the rats to stop eating since the egg white diet was fed. Hurley *et al.* (1982) showed that plasma Zn level of rats was dropped to 45% from the normal level after 8 h of consuming Zn-deficient diet. Even with the early drop of Zn level in rat plasma, food intake was still not affected until four days after which suggests a presence of some regulatory impulse triggered when Zn deficiency reached its critical level that induced the animal to stop eating. Regulatory impulse is more likely stimulated by a change in organ or tissue Zn concentration.

Zn is an important part of about more than 200 metalloenzymes and thus participates in various molecular mechanisms at cellular level and *in vivo* system (Galdes & Vallee, 1983). The present study showed that ALP activity was decreased in plasma ($p<0.05$) of Zn-deficient rats compared with the ZA or PF controls. Also, ALP activities in the liver, kidney and SI were significantly decreased in Zn-deficient rats compared with ZA and PF group, as shown in the plasma ($p<0.05$). Actually, ALP in the plasma is the total amount of alkaline phosphatases released from the tissues such as liver, bone, kidney, intestinal layers and muscle etc, where ALP is synthesized, into the blood. Since no difference in ALP activity between ZA and PF group where food intake should be counted was shown, the decreased plasma ALP activity in Zn-deficient group can be accounted by Zn deficiency, not by decreased food intake. Alkaline phosphatase is a key enzyme for the formation and calcification of the bone tissues. The preosteoblasts differentiate into osteoblasts and then produce ALP, and process procollagens to collagens; and finally the cells form mineralized tissues *in vitro*. (Yamaguchi *et al.,*1986). The decreased blood and tissue ALP activity may be cautiously

interpreted as the potential risk for skeletal growth retardation in rats in the present study.

The Zn concentration in soft tissues such as muscle, brain, liver and skin is not easily mobilized to be available for supporting important metabolic functions (ODell *et al.,* 1989; Roth, 2003). They reported that muscle and liver tissue could not draw upon for mobilization of a Zn deficiency. Our results also supported that tissue Zn level would be mobilized easily even under dietary Zn deficiency. In our study, dietary Zn deficiency did not affect tissue Zn concentration, such as liver, muscle and kidney (Fig. 3). Zn concentration in SI of Zn-deficient rats was significantly affected being lower than ZA rats ($p<0.05$).

Ca, Mg and P are known as major minerals for bone formation and bone mineralization (Koshihara *et al.,* 2005). We analyzed Ca, Mg and P concentration in tissues, such as liver, muscle, kidney, SI and WAT in rats (ZA, PF and ZD). Tissue Ca and Mg concentrations were dependent of dietary Zn level in this study. Ca concentration in tissues, such as liver, muscle, kidney and SI showed consistent pattern depending on different dietary Zn periods. Ca concentrations in liver, muscle and SI of Zn-deficient rats were significantly lower than those of ZA or PF controls ($p<0.05$) (Table 2). Also, kidney Ca concentration was decreased in Zn-deficient rats and unfortunately not shown to be significant. The dietary Zn deficiency of rats lowered Mg concentration more than the ZA rats. Mg concentrations in muscle and SI were significantly dependent on dietary Zn level in this study (Table 2). However, tissue P concentration was not different by dietary Zn groups. Therefore, it can be suggested that dietary Zn deficiency may decrease tissue Ca and Mg concentrations, which may affect negatively skeletal growth in rat model. One unexpected result in this study was that dietary Zn deficiency did not affect WAT Zn, Ca, P and Mg concentrations compared with ZA and ZD ($p<0.05$), rather Zn deficiency group showed lower level of Zn and these minerals, particularly in WA tissue. Further studies are needed to examine whether dietary Zn deficiency affects tissue-specific pattern or some other underlying regulation mechanism. In the present study, pair-fed group was only designed to control the effect of food intake on Zn intake of the Zn-deficient rats. Zn-deficient rats would definitely decrease their food intake and thus decrease Zn and other nutrient intake as well. Pair-fed group was only designed to eliminate this factor.

In summary, the present study demonstrated that ALP and bone related minerals (Ca, Mg and P) are affected by Zn deficiency independent of food intake or body weight. Clearly, rat model shows that Zn is essential to bone health that ALP of dietary ZD rats were significantly decreased in the plasma and minerals, Ca, Mg and P, in the various tissues (liver, muscle and SI) compared to dietary ZA rats. Thus, it can be suggested that dietary Zn deficiency negatively affects bone growth and bone formation in rats.

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