

Zinc Promotes Adipocyte Differentiation *in vitro*

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ABSTRACT : Some researchers reported that beef marbling was improved by the supplementation of organic zinc to a diet satisfying zinc requirement. We studied the relationship between serum zinc concentration and marbling score or serum adipogenic activity in 40 fattened steers. To determine serum adipogenic activities of the steers, preadipocytes were cultured in medium containing the serum samples during differentiation. Although serum zinc concentration was not related to beef marbling score, it was positively correlated to adipogenic activity. Then, we studied the effect of zinc on adipocyte differentiation. Zinc was added into the medium with the similar methods except the addition of fattened calf serum. The activity of glycerophosphate dehydrogenase, a marker of adipocyte differentiation, was significantly increased by the addition of zinc in culture with or without insulin. These results suggest that zinc possibly improved beef marbling through increasing adipogenic activity during fattening. (*Asian-Aust. J. Anim. Sci.* 2001. Vol. 14, No. 7 : 966-969)

Key Words : Zinc, Beef Marbling, Adipogenic Activity, Adipocyte Differentiation, Insulin

INTRODUCTION

Beef marbling is characterized by adipose tissue formation within the skeletal muscle of cattle. The number of adipocytes in the skeletal muscle increased during fattening and the development of beef marbling was closely associated with an increase in adipocyte number within muscle (Cianzio et al., 1985), suggesting that adipocyte differentiation is an important factor on beef marbling.

Zinc is an essential trace mineral and it functions as cofactors of many enzymes and zinc deficiency induces the retardation of growth, anorexia, dermatitis, infertility and dysfunction of immune system (Hongo, 1994). Zinc deficiency is known to affect glucose metabolism through the suppression of insulin production and the reduction of insulin responsiveness (Hongo, 1994). On the other hand, zinc methionine was reported to increase carcass quality of steers (Rust, 1985). Furthermore, the addition of zinc methionine in a diet containing 81 mg/kg zinc was reported to improve carcass quality grade and marbling score of steers (Greene et al., 1988). However, it has not been clarified how zinc increases marbling.

Zinc was suggested to possess insulin like action. May and Contoreggi (1982) reported that zinc stimulate glucose uptake, and inhibited β -agonist enhancing lipolysis in isolated rat adipocytes. Additionally, Shisheva et al. (1992) reported that zinc stimulated lipogenesis in rat adipocytes. They also indicated that dietary supplementation of zinc normalized blood glucose concentration in diabetic rats induced by streptozocin. Since insulin has been known to stimulate adipocyte differentiation, zinc possibly affects beef marbling through its insulin-like action.

We examined the relationship between serum zinc concentration and marbling score or serum adipogenic activity in steers. Additionally, we investigated the effect of zinc on adipocyte differentiation using a mouse preadipocyte, 3T3-L1 cell line.

MATERIALS AND METHODS

Blood was collected from 40 Japanese Black steers at a local slaughterhouse. Serum zinc concentration was measured by an atomic absorption spectrophotometer after digestion with concentrated nitric acid and 60% perchloric acid. The degree of marbling was determined by Beef Marbling Standard of Japan (1988). The adipogenic activity of serum was determined by modification of the method of Torii et al. (1996). The analytical method was as follows.

3T3-L1 preadipocytes, which are derived from mouse 3T3 fibroblast cell line (Green and Kehinde, 1974), were obtained from Dainippon Pharmaceutical Co. Ltd. (Osaka, Japan). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 5% fetal bovine serum (FBS; Bio Whittaker, Walkersville, MD, USA), 100 U/ml penicillin (Sigma, St. Louis, MO, USA) and 100 g/ml streptomycin (Wako Pure Chemical Industries Ltd., Osaka, Japan). After reaching confluence, differentiation was induced in DMEM containing 5% FBS, 0.25 μ M dexamethasone (Sigma, St. Louis, MO, USA), 0.5 mM 1-methyl-3-isobutylxanthine (Sigma, St. Louis, MO, USA) and 10 g/ml bovine insulin (Wako Pure Chemical Industries Ltd., Osaka, Japan) for 48 hours. Thereafter, cells were maintained in DMEM-based media for 6 days as follows. To determine the adipogenic activity in collected serum samples from steers, cells were cultured in medium containing 5 μ g/ml insulin with 10% serum samples. Glycerophosphate dehydrogenase (GPDH) activity was

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measured according to the method of Wise and Green (1979). Protein concentrations were determined according to Lowry's method (1951). The adipogenic activity was expressed as the specific activity of GPDH. Regression analysis was used as statistical analysis.

To determine the effect of zinc on adipocyte differentiation, the similar methods except the addition of fattened calf serum were used. The cells were cultured in medium containing $1 \mu\text{M}$ zinc chloride (Wako Pure Chemical Industries Ltd., Osaka, Japan), 5% FBS with or without 5 g/ml insulin after adipogenic induction. As a marker of adipocyte differentiation, we used specific activity of GPDH. The results were analyzed with two-way ANOVA. All statistical analyses were conducted by GLM procedure of SAS (Statistical Analysis Systems Institute, 1989).

RESULTS AND DISCUSSION

Serum zinc concentration did not relate to beef marbling in fattened steers (figure 1, $p=0.44$). McDowell et al. (1984) suggested that plasma zinc level was below 0.6-0.8 mg/l (approximately 9-12 M) in zinc deficient cattle. Two steers were likely to be mildly deficient in zinc according to this criterion in the present experiment. However, beef marbling score was not low in these steers. These results might suggest that the nutritional status of zinc did not affect beef marbling. On the other hand, serum zinc concentration was positively related ($r=0.62$, $p=0.001$) to serum adipogenic activity in the steers (figure 2).

Although our previous study suggested that serum adipogenic activity was positively correlated to marbling score in beef cattle (Torii et al, 1996), the relationship between serum adipogenic activity and marbling score was not significant in the present experiment (Data is not shown). Cianzio et al. (1985) indicated that the number of intramuscular adipocyte increased during early fattening period, i.e., between 11 and 15 months of age. Beef marbling is possibly affected by serum adipogenic activity when the number of intramuscular adipocyte increases. Greene et al. (1988) reported that beef marbling was improved by the supplementation of organic zinc to a diet satisfying its requirement, but supplementation of inorganic zinc failed to affect beef marbling. Spears (1989) indicated that plasma zinc concentration was higher in sheep given organic zinc than in those given inorganic zinc. It is possible that higher zinc concentration in blood may be favorable for increase of beef marbling in cattle given organic zinc. Therefore, we consider that serum zinc concentration affect beef marbling through increasing serum adipogenic activity in early fattening period.

There are two possible causes how serum zinc level is correlated to adipogenic activity. Zinc in serum directly may regulate adipogenic activity. Otherwise, nutritional status of zinc may affect endocrine factor(s) that regulates adipogenic activity, i.e., the improvement of zinc nutrition increases adipogenic factor(s) in blood, and/or decreases

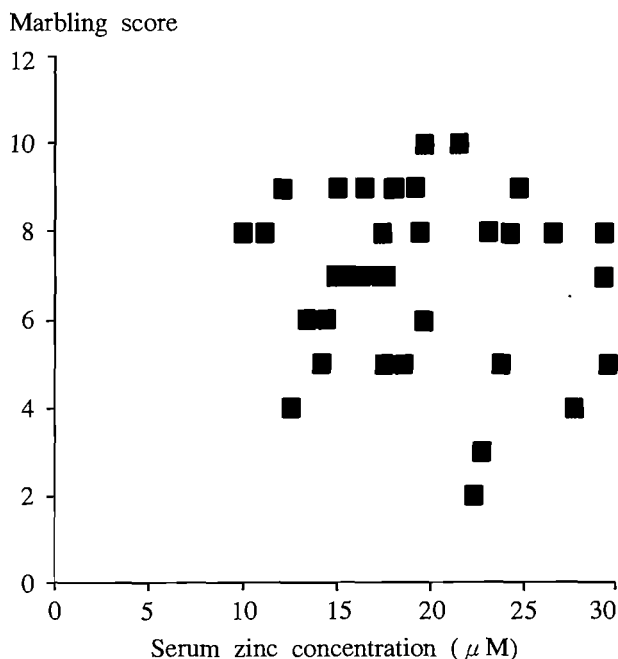


Figure 1. Relationship between serum zinc and beef marbling

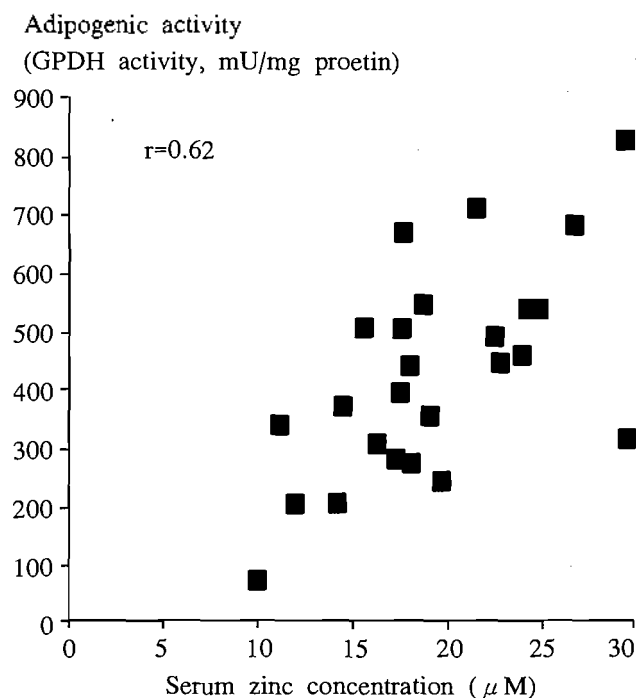


Figure 2. Relationship between serum zinc and serum adipogenic activity

anti-adipogenic factor(s) in blood. Therefore, we examined the addition of zinc to medium for preadipocyte culture to clarify whether zinc directly affected adipogenesis.

The specific activity of GPDH was significantly increased by the addition of zinc at 1 μ M in culture without insulin (figure 3). The enzyme activity was also increased by the addition of insulin alone. The specific activity of GPDH was also significantly increased by the addition of zinc in the presence of insulin. The interaction of zinc and insulin was not significant. We consider that zinc directly stimulates adipogenesis and the action of zinc is additive to that of insulin. In the determination of serum adipogenic activity, the addition of fattened cattle serum increased zinc concentration in medium by 1 μ M-3 μ M. These results suggest that zinc concentration in medium reflects adipogenic activity in cattle serum because the addition of 1 μ M zinc directly stimulated adipogenesis.

Zinc promoted adipogenesis in cultures with or without insulin. Some researchers suggested that zinc had an insulin-like action in rat adipocyte (May and Contoreggi, 1982; Ezaki et al., 1989; Shisheva et al., 1992). On the other hand, Shisheva et al. (1992) reported that the addition of zinc also enhanced the stimulative action of insulin on lipogenesis of rat adipocyte and they suggested that the enhancement was independent of insulin function. Greene et al. (1988) reported that beef marbling was increased by the supplementation of zinc beyond its requirement. Some researchers reported that the supplementation of a large amount of zinc promoted body weight gain in pigs (Fryer et al., 1992; Hahn and Baker, 1993). We consider that zinc beyond its requirement shows

specific function(s) of which mechanism has not been clarified. Further studies are needed to investigate this mechanism. These experiment possibly explain the mechanism of producing highly marbled beef.

CONCLUSION

Zinc promotes adipocyte differentiation *in vitro*. Although serum zinc concentration was not related to beef marbling in the fattened steers, serum zinc might be related to beef marbling during the period when the number of intramuscular adipocytes is increasing.

ACKNOWLEDGEMENT

This work was supported in part by a grant from the Japan Society for the Promotion of Science (JSPS-RFTF97L00905), and by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan (11556053).

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GPDH activity (mU/mg protein)

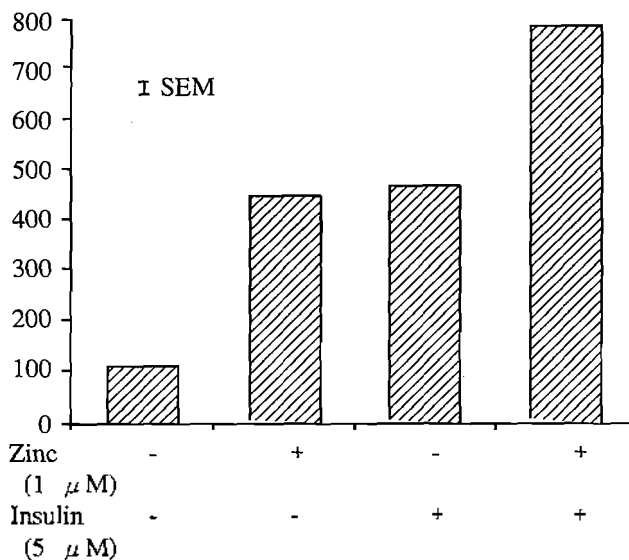


Figure 3. Effect of zinc on adipocyte differentiation

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