Chem. Pharm. Bull. 38(6) 1660—1662 (1990) Vol. 38, No. 6

Alteration of Glucose Consumption and Adenosine Triphosphate Content in Bone Tissue of Rats with Different Ages: The Stimulatory Effect of Zinc

Masayoshi YAMAGUCHI,* Kayoko OZAKI and Yasunobu SUKETA

Department of Environmental Biochemistry and Toxicology, School of Pharmaceutical Sciences, University of Shizuoka, 395 Yada, Shizuoka City 422, Japan. *Received November 13, 1989*

The alteration in bone metabolism at different ages was investigated by estimating glucose consumption and adenosine triphosphate (ATP) content in a culture system of bone tissue from 3- and 30-week-old rats. The femoral-diaphyseal tissue was removed and cultured for periods up to 48 h in Dulbecco's Modified Eagle Medium. Bone tissue was incubated at 37 °C in 5% CO_2/95% air in a medium containing either vehicle and zinc sulfate (10^{-6}—10^{-4} M). The medium glucose consumed by bone tissue clearly increased in a 48 h-culture in 3-week-old rats, while the increase in 30-week-old rats was slight. The presence of zinc sulfate (10^{-6} and 10^{-5} M) caused a significant increase in bone glucose consumption in 3- and 30-week-old rats. ATP content in cultured bone tissue from 30-week-old rats fairly fell in comparison with that from 3-week-old rats. Bone ATP contents in 3- and 30-week-old rats were significantly increased by the presence of zinc (10^{-4} M). The present findings suggest that bone energy metabolism deteriorates with increasing age, and that zinc has a stimulatory effect in elderly rats.

Keywords bone; aging; glucose consumption; ATP production; rat femur; tissue culture

It is known that bone mass decreases in both men and women with increasing age. It is unclear whether the decrease of bone mass is due to increased bone resorption or to decreased bone formation.[1,2] Ovarian hormone deficiency at menopause stimulates bone loss. Current hypotheses have linked ovarian hormone deficiency to defects in the calcium and bone regulatory actions of the calcitropic hormones, calcitonin, 1,25-dihydroxyvitamin D_3, and parathyroid hormone.[3-7] However, none of the current hypotheses receive general acceptance.[8] A possible cause is that the alteration in bone cell function with increasing age is not precisely known. It is important to establish, therefore, the effects of aging on the characteristics of bone metabolism.

More recently, it has been demonstrated that bone formation deteriorates with increasing age, and that the deterioration is partly restored by the oral administration of zinc, which can stimulate bone formation,[9-11] in aged rats.[12] Furthermore, the present study was undertaken to clarify the effect of different ages on bone glucose consumption. We used a culture system to evaluate the function of cells in the bone tissue obtained from weanling and elderly rats. It was found that bone energy metabolism deteriorates with increasing age, and that zinc has a stimulatory effect in elderly rats.

Materials and Methods

Animals Male and female Wistar rats (conventional) were obtained from Japan SLC, Inc., Hamamatsu, Japan. The animals were fed commercial laboratory feed (solid) containing 57.5% carbohydrate, 1.1% calcium, 1.1% phosphorus, and 0.012% zinc at a room temperature of 25 °C, and distilled water freely. The animals were sacrificed at 3 and 30 weeks of age.

Chemicals Dulbecco's Modified Eagle Medium (high glucose, 4500 mg/dl) and a penicillin–streptomycin solution (5000 unit/ml penicillin; 5000 μg/ml streptomycin) were obtained from Gibco Laboratories (Grand Island, NY). Bovine serum albumin (fraction V) was obtained from the Sigma Chemical Co. (St. Louis, MO). Zinc sulfate and all other chemicals were reagent grade from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All water used was glass distilled.

Bone Culture The rats were bled by cardiac puncture under light anesthesia with ether at 3 and 30 weeks of age. The femur was removed aseptically after bleeding and soaked in sterilized ice-cold 0.25 M sucrose solution. The femur was cleaned of soft tissue and marrow, and the diaphysis and epiphysis (containing metaphyseal tissue) were separated. The right femoral-diaphyseal tissues were not cut into small pieces. The femoral-diaphyseal fragments were cultured in a 35-mm dish in 2.0 ml of medium consisting of Dulbecco's Modified Eagle Medium (high glucose, 4500 mg/dl) supplemented with 0.25% bovine serum albumin (fraction V) plus antibiotics (100 units penicillin–100 μg streptomycin/ml of medium).[9] Cultures were maintained at 37 °C in a water-saturated atmosphere containing 5% CO_2 and 95% air for 24 or 48 h. In separate experiments, the respective media contained zinc sulfate.

Analytical Procedures The femoral-diaphyseal fragments were cultured in a medium containing either vehicle or zinc sulfate for 24 or 48 h at 37 °C. After culture, the bone was removed and washed with ice-cold 0.25 M sucrose.

The concentration of glucose in the medium cultured with bone for up to 48 h was determined by the colorimetric method using *o*-toluidine.[13] The dry weight of the bone tissue was measured after extraction with 5.0% trichloroacetic acid, acetone and ether, and ranged from 4.0 to 10.0 mg. The medium glucose consumed by bone culture in 24 or 48 h was expressed as μg of glucose per mg dry acid-insoluble residue of bone tissue. Likewise, the medium pyruvic acid or lactic acid was measured by the enzymatic method.[14] Those data were expressed as μg of pyruvic acid or lactic acid per mg dry acid-insoluble residue of bone tissue.

The adenosine triphosphate (ATP) content in the femoral-diaphyseal tissue was determined by the bioluminescent assay.[15,16] After culture, the bone tissue was immediately frozen, weighed, and then put into boiling 100 mM Tris/3 mM ethylenediaminetetraacetic acid buffer (pH 7.5) for 5.0 min. The boiled bone tissue was cut into small pieces, homogenized with Physcotron homogenizer, and disrupted for 60 s with an ultrasonic device. The supernatant, centrifuged at 10500 × *g* for 10 min, was used for measurement of ATP concentration. The efficiency of ATP extraction was greater than 95%. ATP analysis was reproducible. ATP assay described below was carried out under optimal conditions. An ATP monitoring kit (ATP bioluminescence CLS) was purchased from Boehringer Mannheim-Yamanouchi (Tokyo, Japan). Light emission from the bioluminescent assay was measured in a Luminocounter ATP-237 (Toyo Kagaku Sangyo, Tokyo, Japan). The ATP content of bone tissue was expressed as nmol per g wet bone tissue.

Statistical Methods Data are expressed as the mean ± S.E.M. Statistical differences were analyzed using Student's *t*-test. *p* value of less than 0.05 were considered to indicate statistically significant differences.

Results

The consumption of glucose by bone tissue in rats at 3 and 30 weeks of age was investigated in a culture system for up to 48 h by using the femoral-diaphyseal fragments (Fig. 1). The culture medium glucose was clearly consumed by the bone tissue in the 48 h-culture of 3-week-old rats. However, the consumption in 30-week-old rats was slight.

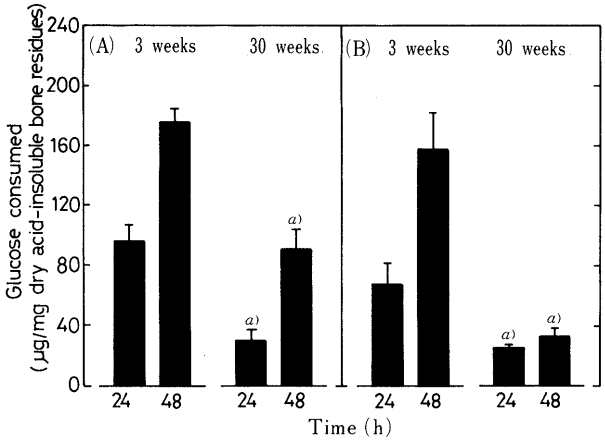

Fig. 1. Alteration of Glucose Consumption in the Femoral Diaphysis of Male (A) and Female (B) Rats of Different Ages

Rats were sacrificed each week. The method of culture with femoral-diaphyseal tissue is described in the text. Each bar represents a mean of five animals. Vertical lines give the S.E.M. *a*) $p<0.01$, as compared with the value of 3-week-old rats.

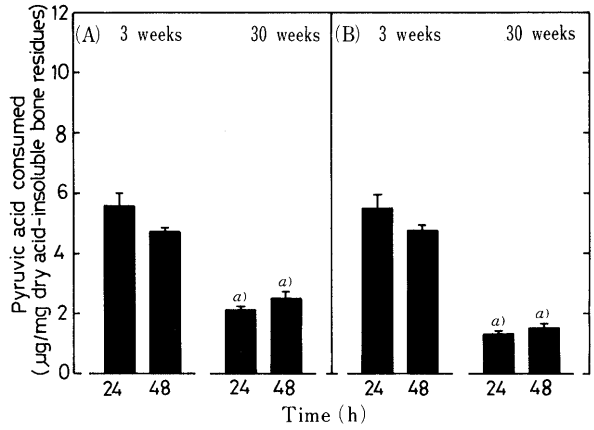

Fig. 2. Alteration of Pyruvic Acid Consumption in the Femoral Diaphysis of Male (A) and Female (B) Rats of Different Ages

Rats were sacrificed each week. The method of culture with femoral-diaphyseal tissue is described in the text. Each bar represents a mean of five animals. Vertical lines give the S.E.M. *a*) $p<0.01$, as compared with the value of 3-week-old rats.

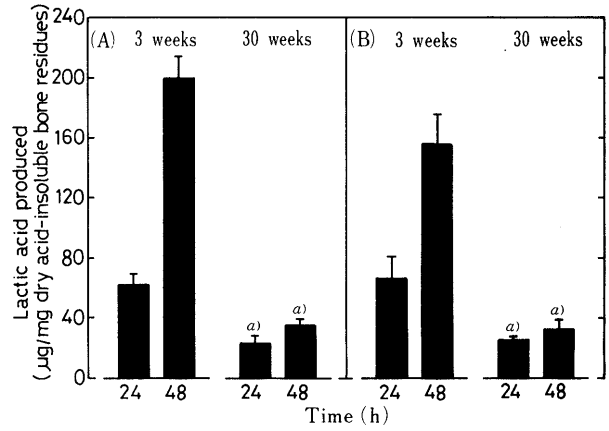

Fig. 3. Alteration of Lactic Acid Production in the Femoral Diaphysis of Male (A) and Female (B) of Different Ages

Rats were sacrificed each week. The method of culture with femoral-diaphyseal tissue is described in the text. Each bar represents a mean of five animals. Vertical lines give the S.E.M. *a*) $p<0.01$, as compared with the value of 3-week-old rats.

The decrease in glucose consumption by bone tissue with increasing age was seen in both male and female rats.

The culture medium pyruvic acid was consumed in a

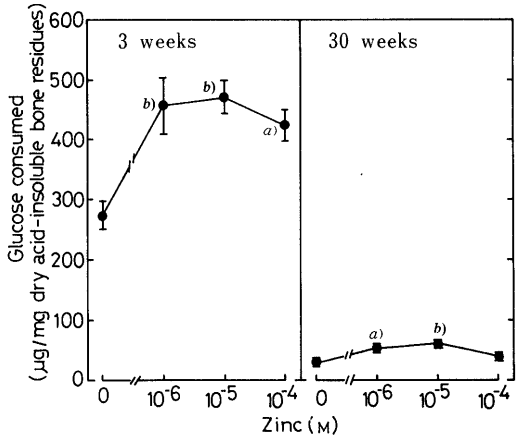

Fig. 4. Effect of Zinc on Glucose Consumption in the Femoral Diaphysis of Male Rats of Different Ages

Rats were sacrificed each week. The method of culture with femoral-diaphyseal tissue is described in the text. The bone tissue was cultured in the presence of either vehicle or zinc sulfate (10^{-6}—10^{-4} M) for 48 h. Each point represents a mean of five animals. Vertical lines give the S.E.M. *a*) $p<0.05$, and *b*) $p<0.01$, as compared with the value of the vehicle alone.

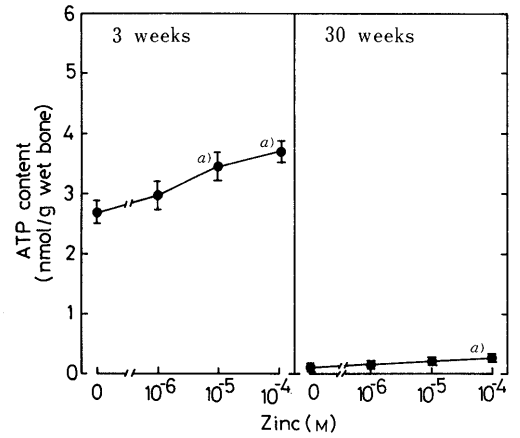

Fig. 5. Effect of Zinc on ATP Content in the Femoral Diaphysis of Male Rats of Different Ages

Rats were sacrificed each week. The method of culture with femoral-diaphyseal tissue is described in the text. The bone tissue was cultured in the presence of either vehicle or zinc sulfate (10^{-6}—10^{-4} M) for 48 h. Each point represents a mean of five animals. Vertical lines give the S.E.M. *a*) $p<0.05$, as compared with the value of the vehicle.

24 h-culture by bone tissue obtained from both male and female rats at 3 and 30 weeks of age (Fig. 2). This consumption was not further enhanced by a 48 h-culture. The consumption of pyruvic acid by bone tissue was slight in 30-week-old male and female rats as compared with that of 3-week-old rats. The consumption of medium pyruvic acid by cultured bone tissue, however, was much less than the glucose consumption.

When the bone tissue of 3-week-old rats was cultured for 24 h, lactic acid was markedly produced from the bones (Fig. 3). This formation was enhanced about 2-fold by a 48 h-culture. In 30-week-old rats, however, the production of lactic acid from cultured bone tissue was weakened.

The effect of zinc sulfate on glucose consumption by bone tissue was investigated in a culture system for 48 h by using the femoral-diaphyseal fragments from male rats of 3 and 30-weeks-old. The presence of zinc sulfate (10^{-6}—10^{-4} M), which can stimulate bone formation,[9,10] in a culture medium caused a remarkable ($p<0.01$) elevation of medi-

um glucose consumption by bone tissue obtained from 3-week-old rats (Fig. 4). The stimulatory effect of zinc (10^{-6} and 10^{-5} M) on bone glucose consumption was also seen in 30-week-old rats. However, the bone response for zinc stimulation in elderly rats was much less than that of weanling rats.

The alteration in the ATP content of bone tissue cultured in the presence of zinc sulfate was examined in a culture system for 48 h by using the femoral-diaphyseal fragments from male rats of 3- and 30-weeks-old. The presence of zinc (10^{-5} and 10^{-4} M) in the culture medium induced a significant increase of ATP content in bone tissue from 3-week-old rats (Fig. 5). The ATP content in cultured bone tissue from 30-week-old rats was reduced in comparison with that of 3-week-old rats. Bone ATP content was significantly increased by the presence of 10^{-4} M zinc. The bone response for zinc in elderly rats was much less than that of weanling rats. When data of bone ATP content was expressed as the amount per bone deoxyribonucleic acid (DNA) (mg), the values were significantly decreased by different ages (data not shown). Also, zinc did not cause a significant alteration of bone DNA content (data not shown).

Discussion

The previous investigation showed that the activities of alkaline and acid phosphatases, DNA and calcium contents in the femoral diaphysis of rats decreased according to increasing age.[12] These decreases were clear in the bone of 28-week-old rats (retired rats) as compared with the bone of 3-week-old rats.[12] The present study, furthermore, was undertaken to clarify the effect of different ages on bone cell functions; glucose consumption and ATP production in bone tissue. This was examined by using a culture system of bone tissue (femoral diaphysis) obtained from 3- and 30-week-old rats. In a 48 h culture, the medium glucose was clearly consumed by the femoral-diaphyseal tissue obtained from 3-week-old rats. This consumption fairly deteriorated in the bone tissue from 30-week-old rats. Such deterioration in bone tissue from elderly rats was seen in both males and females. The consumption of medium pyruvic acid by cultured bone tissue lowered in elderly rats. Also, the production of lactic acid from cultured bone tissue was reduced in elderly rats. The consumption of medium glucose by bone tissue was much more than that of medium pyruvic acid. The production of lactic acid from bone tissue may be related to the consumption of glucose by bone tissue. Furthermore, the ATP content in bone tissue clearly lowered in 30-week-old rats as compared with that of 3-week-old rats. Such a fall of bone ATP content may be based on the lowered glucose consumption, since glucose is important as a source of energy in bone cells.[17] Presumably, energy metabolism linked to glucose consumption deteriorates in bone cells with increasing age.

Bone response for zinc sulfate was also investigated in 3- and 30-week-old male rats. It has been demonstrated that zinc can stimulate bone formation *in vivo*[11] and *in vitro*.[9,10] The presence of zinc sulfate (10^{-6}—10^{-4} M) in culture medium caused a significant increase in glucose consumption by the cultured bone tissue obtained from 3-week-old rats, while the zinc-induced increase in glucose consumption was slight in 30-week-old rats as compared with that in 3-week-old rats. The effect of zinc was remarkable at 10^{-5} M, but it was weakened at 10^{-4} M. Glucose consumption by bone tissue may require a comparatively low concentration of zinc. The present finding suggests that the sensitivity of bone response for the stimulating effect of zinc decreases with increasing age. Moreover, ATP content in cultured bone tissue from 3-week-old rats was increased by the presence of zinc, although the zinc-induced increase in bone ATP content was slight in 30-week-old rats. In this experiment, ATP concentration in the medium after culture with bone tissue from weanling and elderly rats was negligible. This indicates that bone cells were not injured by the culture. Accordingly, the zinc-induced increase in bone ATP content may deteriorate with increasing age, suggesting that bone cell function may lower with increasing age.

The previous investigation showed that the oral administration of zinc sulfate to aged rats could cause a significant elevation of alkaline phosphatase activity and calcium content in bone tissue.[12] In the present study, zinc supplement produced a significant increase in glucose consumption and ATP content in the bone tissue of elderly rats. The present finding further supports the view that zinc plays a nutritional and physiological role as an activator in bone metabolism.

References

1) S. K. Nishimoto, C.-H. Chang, E. Gendler, W. F. Stryker and M. E. Nimi, *Calcif. Tissue Int.*, **37**, 617 (1985).
2) B. E. C. Nordin, J. Aaron and R. C. Crilly, *Lancet*, ii, 277 (1981).
3) R. W. Gray, *Calcif. Tissue Int.*, **33**, 477 (1981).
4) M. Ishida, B. Bulos, S. Takamoto and B. Sacktor, *Endocrinology*, **121**, 443 (1987).
5) S. Morimoto, T. Onishi, T. Okada and Y. Kumahara, *Endocrinol. Jpn.*, **26**, 207 (1979).
6) B. E. C. Nordin, R. Williams, R. H. Marshall, J. C. Gallagher, R. Williams and M. Peacock, *Calcif. Tissue Res.*, **28**, 422 (1976).
7) P. S. Wiske, S. Epstein, N. H. Bell, S. F. Queener, J. Edmondson and C. C. Johnston, *N. Engl. J. Med.*, **300**, 1419 (1979).
8) D. N. Kalu, C.-C. Liu, R. R. Hardin and B. W. Hallis, *Endocrinology*, **124**, 7 (1989).
9) M. Yamaguchi, H. Oishi and Y. Suketa, *Biochem. Pharmacol.*, **36**, 4007 (1987).
10) M. Yamaguchi, H. Oishi and Y. Suketa, *Biochem. Pharmacol.*, **37**, 4075 (1988).
11) M. Yamaguchi and R. Yamaguchi, *Biochem. Pharmacol.*, **35**, 773 (1986).
12) M. Yamaguchi, K. Ozaki and Y. Suketa, *J. Pharmacobio-Dyn.*, **12**, 67 (1989).
13) A. Hyvarimen and E. A. Nikkila, *Clin. Chim. Acta*, **7**, 140 (1962).
14) F. Noll, "Methods of Enzymatic Analysis," Vol. 3, ed. by H. U. Bergmeyer, Academic Press, New York, 1974, pp. 1475—1479.
15) K. Kiyotaka, Y. Kanamoto and Y. Matsuo, *Hiroshima J. Med. Sci.*, **33**, 279 (1984).
16) A. Lundin and A. Thore, *Anal. Biochem.*, **66**, 47 (1975).
17) G. Adamek, R. Felix, H. L. Guenther and H. Fleisch, *Biochem. J.*, **248**, 129 (1987).