Res Exp Med (1990) 190:295–300

Research in
Experimental Medicine
© Springer-Verlag 1990

Aging affects cellular zinc and protein synthesis in the femoral diaphysis of rats

M. Yamaguchi and K. Ozaki

Department of Environmental Biochemistry and Toxicology,
School of Pharmaceutical Sciences, University of Shizuoka,
395 Yada, Shizuoka-City 422, Japan

Received January 10, 1990 / accepted April 23, 1990

Summary. To clarify the effect of aging on bone metabolism, alteration of the cellular zinc content and protein synthesis was examined in the femoral diaphysis of 3- and 30-week-old male rats. The cellular zinc content in bone tissue markedly decreased in 30-week-old compared to 3-week-old rats. When the bone tissue from older rats were cultured with [^{3}H]leucine, incorporation of [^{3}H]leucine into the acid-insoluble residues was less than for weanling rats. This decrease was partly restored by the oral administration of zinc sulfate (0.5, 1.0, and 2.0 mg Zn/100 g body weight) to elderly rats for 3 days. An increase of in vitro [^{3}H]leucine incorporation by bone tissues obtained from the rats that had received zinc (2.0 mg/100 g) was blocked by cycloheximide (10^{-6} *M*) or dipicolinate (10^{-3} *M*), a chelator of zinc. These results suggest that bone protein synthesis declines with age, and that this decline may be based partly on the decrease in bone cellular zinc.

Key words: Aging – Bone metabolism – Protein synthesis – Zinc – Rat femur

Introduction

Serum levels of calcitropic hormones, which regulate bone metabolism, change with increasing age. Serum levels of 1,25-dihydroxyvitamin D_3 decrease during aging [2, 4]. In the mitochondria of kidney [2], 1,25-dihydroxyvitamin D_3 is produced by 1-hydroxylase. This enzyme activity is lowered by aging [5]. In this way, increasing age could induce the decrease of calcium content in bone. Also, with aging, parathyroid hormone immunoreactivity in serum may increase due to a decline in renal clearance of long-lived C-terminal fragments [3, 9]. Additionally, the secretion of calcitonin, which inhibits bone resorption, decreases with age [6].

Alterations in the function of bone cells with increasing age may also contribute to the decrease in bone calcium content. This role, however, is not precisely known. We previously reported that increasing age induced the decline of al-

kaline phosphatase activity and calcium content in the femoral diaphysis of rats [13]. These decreases were partly restored by the oral administration of zinc sulfate and/or 1,25-dihydroxyvitamin D_3 [13]. Previous observations suggested a decrease in bone formation with increasing age. Therefore, the present investigation was undertaken to clarify a possible mechanism for the deterioration of bone formation and calcification with increasing age in rats. It was found that the cellular zinc and protein synthesis decreased in elderly rats. Physiological and nutritional significances of zinc for bone formation in elderly rats were suggested.

Materials and methods

Animals

Male Wistar rats (conventional) were obtained from Japan SLC, Hamamatsu, Japan. The animals were freely fed commercial laboratory chow (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. The animals were killed at 3 and 30 weeks of age.

Chemicals

L-[4,5-^{3}H]Leucine (53.0 Ci/mmol) was obtained from New England Nuclear (Boston, Mass.). Dulbecco's Modified Eagle's Medium (high glucose, 4500 mg/dl) and a penicillin-streptomycin solution (5000 units/ml penicillin; 5000 μg/ml streptomycin) were obtained from Gibco Laboratories (Grand Island, NY). Bovine serum albumin (Fraction V) and cycloheximide were obtained from Sigma (St. Louis, Mo). Zinc sulfate, 2,6-pyridinedicarboxylic acid (dipicolinate), and all other chemicals were reagent grade from Wako Pure Chemical Industries (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, cleaned of soft tissue and marrow, was completely removed by washing, 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 medium consisting of Dulbecco's Modified Eagle's 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) [11]. Cultures were maintained at 37°C in a water-saturated atmosphere containing 5% CO_2 and 95% air for 2 or 4 h.

In separate experiments, rats (30-week-old) received an oral administration of zinc sulfate (0.5, 1.0, and 2.0 mg Zn/100 g body weight) for 3 days, and the animals were killed 24 h after the last administration. The femoral diaphysis was obtained aseptically and cultured in medium containing either vehicle (sterile distilled water), cycloheximide (10^{-6} *M*), or dipicolinate (10^{-3} *M*) for 2 h.

Bone protein synthesis

Effect on newly synthesized bone total protein was determined by studying the incorporation of [^{3}H]leucine [1, 12]. The diaphyseal fragments were pulsed with [^{3}H]leucine (5.0 μCi/ml of medium) at the initiation of culture, and cultured for 2 or 4 h. At the end of the culture, the bone tissue was removed and washed with ice-cold 0.25 *M* sucrose. Bone tissues were extracted with ice-cold 10% trichloroacetic acid, acetone, and ether, and then rinsed in ice-cold 0.25 *M* sucrose. The bones were dried and weighed. To determine the amount of [^{3}H]leucine incorpo-

ration into bone total protein, the dried bone residues were dissolved in 1.0 ml of 0.2 *N* NaOH, and an aliquot was removed and placed in a vial to measure the disintegrations per minute by scintillation counting. Data are expressed as disintegrations per minute (dpm) per mg dry weight of acid-insoluble residues.

Bone zinc content

Diaphyseal fragments were removed and washed with ice-cold 0.25 *M* sucrose solution, blotted, and weighed. The bone tissue was minced in 2.0 ml of 0.1 *N* NaOH solution and shaken for 24 h at 4°C [12]. The bone tissue (matrix) remaining after alkaline extraction was separated by centrifugation at 600 *g* for 5 min, and the supernatant fractions (cellular components) were retained. Zinc content in the bone matrix and the cellular component was determined by atomic absorption spectrophotometry after digestion with nitric acid. Bone zinc content was expressed as micrograms of zinc per gram of wet bone tissue.

Statistical analyses

Data are expressed as means ± SEM. Statistical differences were analyzed using Student's *t*-test. *P*-values of less than 0.05 were considered to indicate statistically significant differences.

Results

Effect of aging on zinc content in the femoral diaphysis of rats is shown in Fig. 1. Zinc content in the bone matrix of elderly rats (30 weeks old) did not differ from weanling rats (3 weeks old). However, zinc content in the cellular components of bone tissues decreased significantly with age. The total amount of zinc in bone tissues (matrix and cellular components) did not alter significantly with increasing age.

Alteration of in vitro protein synthesis in the femoral diaphysis of different aged rats is shown in Fig. 2. The effect on newly synthesized bone total protein was determined by measuring the incorporation of [^{3}H]leucine into the cultured diaphyseal fragments. Incorporation of [^{3}H]leucine for 2 or 4 h into the bone tissues from elderly rats was lower than the incorporation into bone tissue from weanling rats. Also, [^{3}H]leucine incorporation per DNA (mg) of bone tissue significantly decreased with increasing age (data not shown).

The effect of oral zinc administration on protein synthesis in the cultured femoral diaphysis fragments from elderly rats was examined. Incorporation of [^{3}H]leucine significantly increased in the bone tissue at all doses of zinc sulfate (0.5, 1.0, and 2.0 mg Zn/100 g), as shown in Fig. 3.

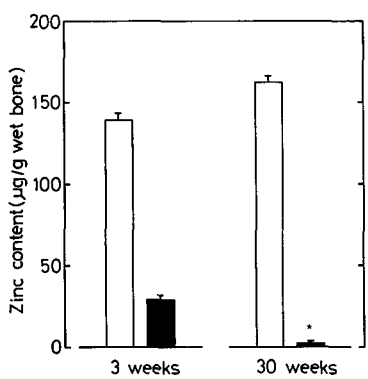

Fig. 1. Alteration of zinc content in the femoral diaphysis of rats of different ages. Each *bar* is the mean for five rats. *Vertical lines* represent the SEM. * $P < 0.01$ compared with the value for 3-week-old rats. □, Bone matrix zinc; ■, bone cellular zinc

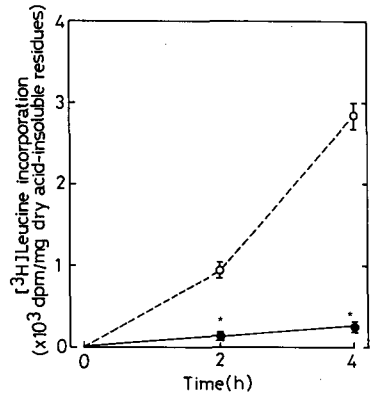

Fig. 2. Alteration of in vitro protein synthesis in the femoral diaphysis of rats of different ages. Femoral-diaphyseal fragments were cultured in medium containing [^{3}H]leucine. Each *point* is the mean for five rats. *Vertical lines* represent the SEM. * $P < 0.01$ compared with the value for 3-week-old rats. ○----○, 3-week-old rats; ●——●, 30-week-old rats

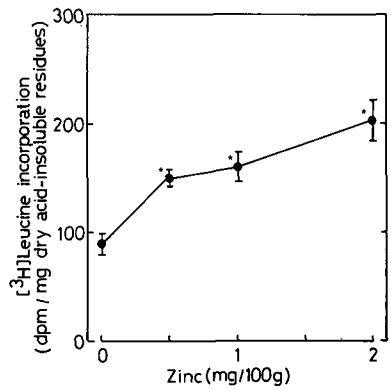

Fig. 3. Alteration of in vitro protein synthesis in the femoral diaphysis of elderly rats treated with zinc sulfate p.o. Animals were killed 24 h after conclusion of the zinc administration (3 days). The femoral-diaphyseal fragments were cultured in medium containing [^{3}H]leucine for 2 h. Each *point* is the mean for five rats. *Vertical lines* represent the SEM. * $P < 0.01$ compared with the control values

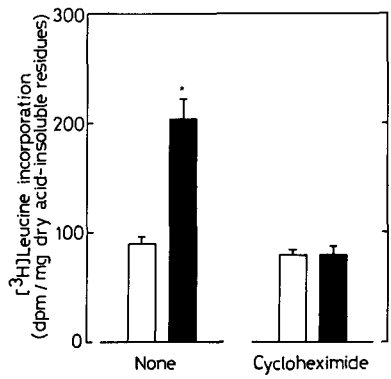

Fig. 4. Effect of cycloheximide on the zinc administration-induced increase of in vitro protein synthesis in the femoral diaphysis of elderly rats. The rats received the zinc sulfate (2.0 mg Zn/100 g) for 3 days, as in Fig. 3. Femoral-diaphyseal fragments were cultured in medium containing [^{3}H]leucine and cycloheximide for 2 h. Each *bar* is the mean for five animals. *Vertical lines* represent the SEM. * $P < 0.01$ compared with the control value. □, Control; ■, bone of zinc-treated rats

When cycloheximide, an inhibitor of protein synthesis, was added to the culture medium, the zinc-induced increase in the incorporation of [^{3}H]leucine into bone tissue was blocked completely (Fig. 4). Thus, the administration of zinc caused an increase of protein synthesis in bone tissues of elderly rats.

Dipicolinate is a chelator of zinc [7, 8]. Diaphyseal fragments obtained from elderly rats that had received zinc sulfate were cultured for 2 h in medium con-

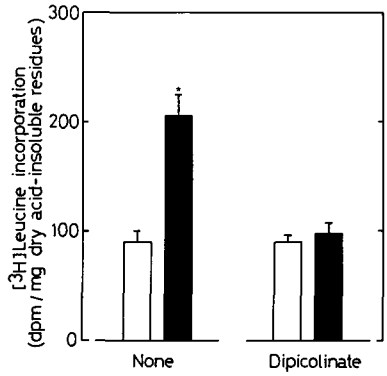

Fig. 5. Effect of dipicolinate, a chelator of zinc, on the zinc administration-induced increase of in vitro protein synthesis in the femoral diaphysis of elderly rats. The rats received zinc sulfate as in Fig. 4. Femoral-diaphyseal fragments were cultured in medium containing [^{3}H]leucine and dipicolinate for 2 h. Each *bar* is the mean for five animals. *Vertical lines* represent the SEM. $*P < 0.01$ compared with the control value. □, Control; ■, bone of zinc-treated rats

taining dipicolinate. The presence of dipicolinate completely blocked the increase of [^{3}H]leucine incorporation in bone tissues (Fig. 5).

Discussion

Alterations in the function of bone cells with increasing age are not precisely known, although it has been reported that renal production of 1,25-dihydroxyvitamin D_3, which regulates bone metabolism, declines with increasing age [5]. Our previous investigation demonstrated that bone metabolism in male and female rats altered with the passage of time; increasing age induced decreases in alkaline and acid phosphatase activities, deoxyribonucleic acid (DNA), and calcium contents in the femoral diaphysis of rats [13]. These decreases were clearly seen at 28 weeks of age as compared with bone metabolism of 3-week-old rats [13]. The age-induced decrease of bone metabolism was partly restored by the oral administration of zinc and/or 1,25-dihydroxyvitamin D_3 to elderly rats (28 weeks old) [13]. The mechanism by which the function of bone cells deteriorates with increasing age, however, remains to be elucidated. The present investigation, therefore, was undertaken to clarify a possible mechanism for altering bone metabolism with increasing age.

Zinc content in the cellular components, but not the matrix, was lower in the femoral diaphysis of elderly rats (30 weeks old) than in that of weanling rats (3 weeks old). Recently, it has been demonstrated that zinc, an essential trace metal, plays a physiological role as an activator of bone formation in weanling rats; the metal can stimulate protein synthesis at the translational level in bone cells in vivo [10] and in vitro [11, 12]. Presumably, the decrease in bone celular zinc content is of importance in the development of bone metabolism deterioration with increasing age.

[^{3}H]Leucine incorporation into the acid-insoluble residues of bone tissues from 30-week-old rats markedly decreased compared to 3-week-old rats. Therefore, bone protein synthesis most likely deteriorates with increasing age. Oral administration of zinc sulfate (0.5, 1.0, and 2.0 mg Zn/100 g body weight) for 3 days partly restored the rate of leucine incorporation. Presumably, the increase in bone cellular zinc content caused by zinc administration induced the stimulation of bone protein synthesis. Cycloheximide, an inhibitor of protein synthesis at the translational process, completely blocked the zinc administration-induced increase in [^{3}H]leucine incorporation into the bone tissue obtained from elderly

rats. This result supports the view that the zinc administration-induced increase in [^{3}H]leucine incorporation into the bone tissue is based on the stimulated bone protein synthesis.

Dipicolinate (2,6-pyridinedicarboxylic acid), a chelator of zinc, has been used to prevent the effect of zinc on enzyme activity [7, 8]. The zinc chelator presumably decreases the zinc effect mainly by forming an extracellular Zn (dipicolinate)$_2^{2-}$ complex [7]. Dipicolinate in culture medium completely blocked the increase in [^{3}H]leucine incorporation into the bone tissue obtained from elderly rats treated with zinc. This finding supports the view that the zinc-induced increase in bone protein synthesis may result from the increased level of zinc in bone cells. Thus, zinc has a stimulatory effect on bone protein synthesis in elderly rats.

It has been reported that serum levels of calcitropic hormones, which stimulate bone formation, decrease with increasing age [3, 4, 9]. Deterioration of bone protein synthesis with increasing age may be involved in the decrease of serum calcitropic hormone levels. However, the present investigation clearly demonstrates that increasing age causes the decrease of cellular zinc content and protein synthesis in bone tissue, and that the decreased bone protein synthesis is partly restored by the supplement of zinc. From the present findings, it appears that the decrease in bone cellular zinc content induces the deterioration of bone protein synthesis with increasing age. The depletion of zinc in bone cells may induce the deterioration of bone formation in elderly rats. Zinc presumably plays a physiological role in the development of bone disorder with increasing age. In aging, the supply of zinc may be important in preventing bone disorder.

References

1. Canalis E (1985) Effect of sodium vanadate on deoxyribonucleic acid and protein synthesis in cultured rat calvaria. Endocrinology 116:855–862
2. DeLuca HF (1979) Vitamin D: metabolism and function. Springer, Berlin Heidelberg New York, pp 1–80
3. Fugita T, Okano K, Orimo H, Ohata M (1972) Age and fate of parathyroid hormone. J Gerontol 27:25–27
4. Gray RW (1981) Effects of age and sex on the regulation of plasma 1,25$(OH)_2D_3$ by phosphorus in the rat. Calcif Tissue Int 33:477–484
5. Ishida M, Bulos B, Takamoto S, Sacktor B (1987) Hydroxylation of 25-hydroxyvitamin D_3 by renal mitochondria from rats of different age. Endocrinology 121:443–448
6. Morimoto S, Onishi T, Okada Y, Kumahara Y (1979) Comparison of human calcitonin secretion after a 1-minute calcium infusion in young normal and elderly subjects. Endocrinol Jpn 26:207–211
7. Pocker Y, Fong CTO (1980) Kinetics of inactivation of erythrocyte carbonic anhydrase by sodium 2,6-pyridinedicarboxylate. Biochemistry 19:2045–2050
8. Rognstad R (1984) Inhibition of glycogen synthesis in rat hepatocytes by medium Zn^{2+}. Biochem Biophys Res Commun 122:726–733
9. Wiske PS, Epstein S, Bell NH, Queener SF, Edmondson J, Johnston CC (1979) Increases in immunoreactive parathyroid hormone with age. N Engl J Med 300:1419–1421
10. Yamaguchi M, Yamaguchi R (1986) Action of zinc on bone metabolism in rats: increases in alkaline phosphatase activity and DNA content. Biochem Pharmacol 35:773–777
11. Yamaguchi M, Oishi H, Suketa Y (1987) Stimulatory effect of zinc on bone formation in tissue culture. Biochem Pharmacol 36:4007–4012
12. Yamaguchi M, Oishi H, Suketa Y (1988) Zinc stimulation of bone protein synthesis in tissue culture: activation of aminoacyl-tRNA synthetase. Biochem Pharmacol 37:4075–4080
13. Yamaguchi M, Ozaki K, Suketa Y (1989) Alteration in bone metabolism with increasing age: effects of zinc and vitamin D_3 in aged rats. J Pharmacobiodyn 12:67–73