Running at 0.7 km/h for 10 min every day inhibited development of osteoporosis caused by protein-deficient (PD) food intake. Urine alkaline phosphatase (ALP), a marker of bone formation osteoporosis, was not elevated in rats fed PD, when the osteoporosis was inhibited by running. Estrogen supplementation increased bone-breaking energy (BBE), but did not increase bone mineral density (BMD), and did not decrease urinary ALP levels.

**Key words:** osteoporosis; protein-deficient food; running; estrogen; alkaline phosphatase

In general, osteoporosis is classified into primary and secondary osteoporosis. The former is further divided into type I and type II. Type I includes postmenopausal osteoporosis and is also called the bone resorption type. Type II includes evolutional osteoporosis and is called the bone formation type. Desoxypyridinoline (Dpd) is used as a marker of type I osteoporosis and alkaline phosphatase (ALP) as a marker of type II. Rats fed a restricted diet containing 50% less in carbohydrates and oil but normal levels of protein, minerals and vitamins show osteoporosis with significant reductions of BMD and BBE and an increase in femoral X-ray density. Because the feces show high concentrations of Dpd, this appears to be similar to type I osteoporosis.

Estrogen and exercise are reported to inhibit the development of osteoporosis after ovariectomy, postmenopausal osteoporosis, and osteoporosis caused by restricted food intake. Rats fed a PD diet are reported to have osteoporosis with significant decreases of BBE and BMD. Because urinary ALP activity was observed as a marker of the bone formation type, this form of the osteoporosis is considered to be similar to type II.

The efficacy of exercise and estrogen against type II osteoporosis and osteoporosis induced by PD food intake is unknown. Hence, this study was undertaken to determine whether osteoporosis caused by PD food intake can be ameliorated by estrogen and exercise.

Female rats (6 weeks old) of the Wistar strain were kept on standard feed prepared according to the AIN-93G for one week before the start of the experiment. The animals were then separated into 4 groups of 5 rats each. Group 1 was fed the standard AIN-93G feed, Group 2 was fed PD food with sugar in place of the protein in the standard feed, and Group 3 was fed PD food and forced to run 10 min/day at 0.7 km/h on a treadmill, as reported previously. Group 4 was fed PD feed with 2.50 μg of conjugated estrogen every day (calculated by converting 0.625 mg of estrogen per unit weight of a 50 kg woman to an equivalent amount of estrogen per unit weight (100 g) of a rat and doubled to increase the effect).

The body weight and food intake of each animal were measured every day. The animals were killed after 3 weeks and blood was drawn. Serum was collected by centrifuging the blood at 12,000 rpm for 20 min. The liver, kidney, adrenal gland, and uterus were then removed and their total weights were measured. The right and left femurs were removed and stored at -60°C.

The Ryukyu University Guideline for the Care and Use of Laboratory Animals was followed. BBE, BMD, and the density of X-ray images were measured as described in previous papers. Estradiol concentration in the serum was measured with an ELA kit (Funakoshi). ALP activity in urine was determined with Wako kits.

Data were tested by one-way analysis of variance followed by inspection of differences between means by Duncan’s new multiple range test throughout these experiments. The different superscript letters in the figures show statistically significant differences at p < 0.05.

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Abbreviations: PD, protein-deficient; ALP, alkaline phosphatase; BMD, bone mineral density; BBE, bone breaking energy; Dpd, desoxypyridinoline
The body weight of Group 1, fed standard food, was about 120 g at the start of the experiment and gradually increased to 145 g, with an average increase of 24.2 g over 3 weeks. The weights of Groups 2, 3, and 4 fell step by step to about 93 g with a decrease of 29 g, 95 g for a decrease of about 24 g, and 91.0 g for a decrease of 28 g respectively. The real values of the gain or loss were 24.2 g ± 7.2, a -28.9 g ± 4.7, a -24.4 ± 2.9, a and -28.6 g ± 2.5, b in Groups 1, 2, 3, and 4 respectively. The food intakes of Groups 1, 2, 3, and 4 were 192 g ± 20.9, a 151.8 g ± 12.2, b 167.6 g ± 33.4, b and 149.0 g ± 8.1 b for 3 weeks, respectively. Rats fed PD food had poor appetite and lost weight. The heart weights of Groups 2–4, rats fed PD food, were significantly high compared with Group 1. Spleen weights decreased significantly in Groups 2–4 in contrast with Group 1. The weights of liver, kidney, pancreas, and adrenal gland were the same in all groups.

The values for BBE (toughness, 10^-3 J) of the femur in Groups 1, 2, 3, and 4 were 28.49 ± 3.57 a, 21.32 ± 4.58 b, 23.83 ± 0.44 a, and 23.83 ± 1.83 a respectively. Group 2, which was fed PD food, showed significantly lower BBE values compared with Group 1 rats. The figures for Group 3 and Group 4 rats were significantly higher than those for Group 2.

The BMD of Group 2 and Group 4 rats showed significantly lower values in contrast with that of Group 1 rats. The values for Group 3 rats were high compared with those for Groups 2 and 4, but showed no significant difference from those for Group 1. The BMD values (g/cm^2) for Groups 1, 2, 3, and 4 were 0.057 ± 0.02 a, 0.017 ± 0.01 b, 0.037 ± 0.01 a, and 0.032 ± 0.01 b respectively. (Fig. 1)

In X-ray images, bone rich in calcium is white due to X-ray absorption. The values for the density of bone X-rays in Group 1, 2, 3, and 4 were 0.34 ± 0.03 a, 0.82 ± 0.04 b, 0.43 ± 0.04 a, and 0.86 ± 0.08 b respectively. The densities for Groups 2 and 4 were considerably higher than those for Group 1. Group 3 was almost the same as Group 1, but lower than Group 2. Hence, Group 2 rats were considered to have osteoporosis, a condition in which bones break easily. Running was found to inhibit the development of osteoporosis caused by PD feed intake. On the other hand, estrogen was efficacious against BBE, but ineffective in the results of BMD and X-ray density. Further study is needed to clarify the efficacy of estrogen against osteoporosis.

The uterus weight values per 100 g body weight for Groups 1, 2, 3, and 4 were 0.30 ± 0.04 a, 0.20 ± 0.02 b, 0.21 ± 0.05 b, and 0.37 ± 0.04 a respectively. Uterus weights for Groups 2 and 3 were significantly lower than those for Groups 1 and 4. The serum estradiol values (pg/ml) were 58.93 ± 14.55 a, 28.48 ± 7.14 b, 36.36 ± 5.38 a, and 57.40 ± 4.00 b for Groups 1, 2, 3, and 4 respectively. The levels for Groups 1 and 4, which were fed PD food with estrogen, increased compared with those of Groups 2 and 3. Because uterine weight is known to correlate with concentration of estradiol in serum, the results for uterine weight agreed with those for serum estradiol. Womb weight and level of serum estradiol did not appear to be connected with inhibition of osteoporosis.

The urinary ALP activities (KA) were 6.60 ± 1.02 a, 29.48 ± 3.42 b, 19.59 ± 2.71 c, and 24.73 ± 9.65 b for Groups 1, 2, 3, and 4 respectively. (Fig. 2) Values for Groups 2 and 4 were significantly higher than those for Group 1. The level for Group 3 was lower than those for Groups 2 and 4, but higher than those for Group 1. The results for urinary ALP correlated well with those for femoral BMD, BBE, and X-ray density in rats fed PD food with running and rats fed standard food.

References
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**Fig. 1.** Bone Mineral Density (BMD) of the Femur. Group 1 was fed standard food prepared according to AIN-93G, Group 2 was fed protein-free food, Group 3 was fed protein-free food caused to run 0.7 k/h for 10 min every day and Group 4 was fed protein-free food containing 2.5 μg of estradiol every day. Mean ± SD (n = 5) Values not sharing common superscript letters (a and b) are significantly different at p < 0.05.

**Fig. 2.** Alkaline Phosphatase Activity in Urine. Mean ± SD (n = 5) Values not sharing common superscript letters. (a, b, and c) are significantly different at p < 0.05.


