Resistance Running Exercise Effectively Prevents Bone Loss in Ovariectomized Rats

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Key words: ovariectomized rat, resistance running exercise, endurance exercise, bone mineral density (BMD), bone metabolic marker

Abstract

In the present study, we examined effects of difference of endurance and resistance running on bone mineral density of ovariectomized SD rats. Thirty 12wk-old female SD rats were assigned to four groups: 1) sham operated (Sham); 2) ovariectomized (OVX); 3) OVX endurance exercised (OEN); 4) OVX resistance running exercised (ORE). All rats were fed a low Ca (0.1%) diet ad libitum. Endurance exercising rats were forced to run 40 minutes on a uphill (7%) treadmill at 21m/min. Resistance running exercising rats were forced to take 40 sets of 1 min run interspersed with 1min rest with a 100g weight on the back on a uphill (11%) treadmill at 21 m/min. The experimental duration consisted of the 2 weeks adaptation periods and 4 weeks treatment periods. The bone mineral density (BMD) of femur, tibia and fourth lumbar vertebrae (L4) in OVX, assessed by DEXA (Lunar) and tibial trabecular volumetric BMD assessed by pQCT (Stratec) were obviously reduced ($p<0.01$) than Sham rats. Resistance running exercise significantly prevented the bone loss caused by ovariectomy in ORE rats. Urinary excretion of deoxypyridinoline-adjusted creatinine (DPD) in OVX significantly increased compared with Sham ($p<0.05$). Although DPD of OEN significantly higher than Sham, that of ORE tended to lower than OVX,. These results suggested the possibility that suitable resistance running exercise have more beneficial effects than endurance exercise on alleviating bone loss of ovariectomized rats and this preventing effects of bone loss were caused by suppress of bone resorption.

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Introduction

Estrogen deficiency as in postmenopause and ovariectomy promptly induces bone loss, increasingly developing to osteoporosis. Loading mechanical stresses are well elucidated to restrain bone metabolic turnover and result in maintaining bone mass (Frost[8], 1992). There have been presented evidences in rats regularly running on a treadmill supporting Frost's proposal (Yeh et al[24]., 1993; Barengolts[3] et al., 1994). Barengolts[2] et al. (1993) reported endurance exercise (21m/min, 7 % inclination, 40min/day, 4days/week for 3 month) increased the ash weight of femurs and

tibia. Another various exercises including jump (Umemura[20] et al., 1995), squat exercises training (Westerlind[22] et al., 1998), tower climbing exercise (Notomi[18] et al., 2001), have been examined for the influences on bones and proved to effectively improve their status. Resistant exercise training directly stimulates bones and is assumed to act more effectively for increasing bone masses as compared to endurance training (Heinrich[10] et al., 1990; Bennell[4] et al., 1997; Snow-Harter[19] et al., 1992). Nakajima[17] et al. (2001), we originated new type of resistance exercise, which stimulated bone by climbing uphill with loading on the back and showed that the exercise increased BMD of femur and fourth lumbar vertebrae in ovariectomized rats. The details of effects, however, the resistance exercise on the prevention of bone loss in estrogen deficiency remains to be solved.

This study was designed to examine the inhibitory effects of this resistance exercise on OVX-induced bone loss as compared with endurance exercise model referred to Barengolts[2] et al. (1993)

Materials and methods

Animal care

Female Sprague-Dawley rats aged 12 weeks were purchased from Japan Charles River Co., Ltd. The animals were individually housed in a room maintained at about 24°C and 50% humidity with 14h/10h light-dark cycle. They were sham or ovariectomy (OVX) operated within several days after arrival, and randomly assigned to four groups on the body weight basis: (A) sham-operated (Sham; n=4), (B) ovariectomized (OVX; n=6), (C) OVX endurance running exercised (OEN; n=6), (D) OVX resistance running exercised (ORE; n=6). Rats were fed on a low calcium (0.1%) powdered diet prepared according to the AIN-93M prescription for six weeks including initial two weeks of the adaptation period. The body weight and food intakes were recorded once a week. At the end of experimental period, rats were anesthetized with pentobarbital and exsanguinated to death. The femur, tibia and fourth lumbar vertebrae (L4) were removed and the bone status was measured. Uterine, adrenal and abdominal fat depots were weighed.

Exercise training

Rats in OEN and ORE groups were accustomed to run on a motor-driven treadmill (KN-73, Natsume, Japan) with an electric grid at the rear of each compartment for the third week after ovariectomy (14week old). During a week of training rats began to practice walking at the speed of 6m/minute without the inclination of the treadmill, 10 minutes/day, and then each groups were trained to be able to run at experimental protocols (**Fig. 1**). In the endurance running group, speed and grade gradually raised and kept to run at 21m/min with 7% uphill grade of 40 minutes (Barengolts[2] et al.; 1993) for four weeks. ORE rats were forced to run at 21m/min with 11% uphill grade by the gradual increase of the speed and grade of the treadmill. After a week of practicing the exercising rats were regularly taken 40 sets of intermittent 1-min run interspersed with 1-min rest, loading 100g weight on the back, 5 days a week for further four weeks (Nakajima et al[17]., 2001). All procedures were in accordance with the Waseda University Guidelines for the Care and Use of Laboratory Animals.

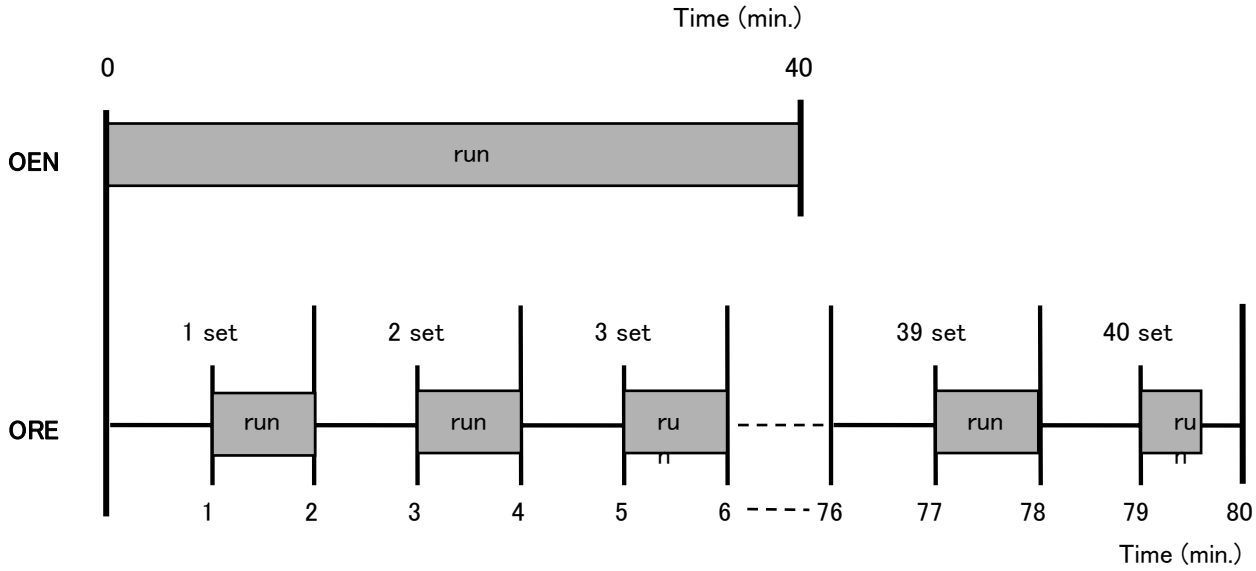

Fig. 1 Protocol of running training on the treadmill. The OEN group was continuously forced to run for 40 minutes on a uphill 7% treadmill at 21m/min. The ORE animals were forced to run on a treadmill at 21m/min, 11% grade, 5 days/week. Forty-sets of run and rest for 1 minute, respectively, was performed with loading of 100g weight on the back. OEN; OVX + endurance running exercise, ORE; OVX + resistance running exercise.

Bone status and bone metabolic marker measurement

The femur, tibia and L4 were freed of soft tissue. Bone mineral density (BMD) was estimated using dual energy X-ray absorptiometry (DEXA, Lunar DPX-L, USA). Cortical and trabecular volumetric bone mineral density (BMD) at distal metaphysis and mid-shaft of tibia (3.0 or 12.0mm below the proximal joint end of the tibia, respectively) was measured by peripheral quantitative computed tomography (pQCT, XCT960A, Stratec, Germany). Serum alkaline phosphatase (ALP, IU/l) as bone formation marker was measured by methods of phenyl phosphate using ALP-K test kits (Wako, Japan) according to the manufacturer's instruction. 24 hours urinary excretion of Deoxypyridinoline adjusted Creatinine (Dpy, Nmol/Mmol CRE) as bone abroption marker was measured by methods of EIA using Osteolinks-DPD test kits (Sumitomo, Japan).

Statistical analysis

Data were expressed as means±SE. The significance of the differences was determined by ANOVA and Tukey-kramer test. Difference were considered significant at the level of $p<0.05$.

Results

Change of body weight and organ weights

When the adaptation period started at 12 week old, the average body weights of all the groups were practically the same. At the final week of the treatment, the body weight of OVX group was approximately 18% higher than that of Sham ($p<0.05$) (**Table 1**). A gain of body weight in OVX, which was difference between initial and final body weight, was huge increase (160%) compared with Sham ($p<0.05$). With endurance or resistance exercise lasted for 5 weeks, included one week of adaptation periods, the weight gain was somewhat depressed in two exercising groups, OEN and ORE. The average daily food intake of two exercising

groups was significantly lower than that of OVX ($p<0.05$).

Uterine weight in OVX, OEN and ORE was markedly reduced to approximately 17% of that in Sham ($p<0.001$). The abdominal fat weight was decreased in three OVX operation groups as compared to that in Sham ($p<0.01$).

Table 1 Effects of resistance and endurance running exercise on body and organs weight in rats.

	Sham	OVX	OEN	ORE
n	4	6	6	6
Body weight (g)				
12 week old (initial weight)	253±7	248±7	250±6	244±3
15 week old (experiment start)	278±11	329±10**	296±11##	297±5#
18 week old (final weight)	294±9	339±17	314±16	293±19#
Gains of body weight (g)	34.9±10.1	91.1±20.0*	64.5±17.2	49.3±17.7
Food intake (g/day)	16.2±0.4	17.5±0.8	15.2±0.8#	14.9±0.5#
Organs weight (g/100g BW)				
Uterus	0.179±0.012	0.030±0.002***	0.031±0.003***	0.032±0.004***
Abdominal fat	3.37±0.47	1.81±0.23**	1.63±0.20**	1.27±0.24**

Sham; sham operated, OVX; ovariectomized, OEN; endurance exercise with OVX, ORE; resistance exercise with OVX. OVX was performed at 12 weeks, and exercises treated from age of 15 to 18 weeks. Values are mean±S.E. **, *** : $p< 0.01, 0.001$ vs. Sham, #, ## : $p<0.05, 0.01$ vs. OVX.

Effects on bone status in femurs, tibias and fourth lumbar vertebrae (L4) and bone metabolic markers

The femoral BMD of OVX measured by DEXA were significantly declined as compared to that of Sham ($p<0.001$, **Fig. 2**). Those of tibia and L4 were similar tendency to that of femur ($p<0.01$, 0.001). There was no significant difference between Sham and ORE groups in tibia. Resistance running exercise induced a slight but significant increase in femur ($p<0.05$), tibia ($p<0.01$), and L4 ($p<0.05$) as compared to OVX. There was no BMD (g/cm^3) loss measured by pQCT in cortical bone of tibia in OVX compared to Sham, and exercise training increased compared to Sham in both OEN and ORE (**Fig. 3**). On the other hand, trabecular BMD of tibia measured by pQCT showed significantly decrease caused by OVX as compared to Sham ($p<0.001$). Resistance running exercise significantly increased BMD compared to that of OVX ($p<0.01$). Serum ALP (IU/l; mean±SE) in Sham and OVX were 34.23±4.25 and 63.62±7.44, respectively (Fig. 4). OVX significantly increased Serum ALP ($p<0.01$), and similar tendency was shown in ORE ($p<0.05$). 24 hours urinary excretion of Deoxypyridinoline adjusted Creatinine (Dpy, Nmol/Mmol CRE) in Sham and OVX were 106.6±7.1 and 171.5±18.8, respectively. OVX significantly increased Deoxypyridinoline ($p<0.05$), and similar tendency was shown in OEN ($p<0.05$).

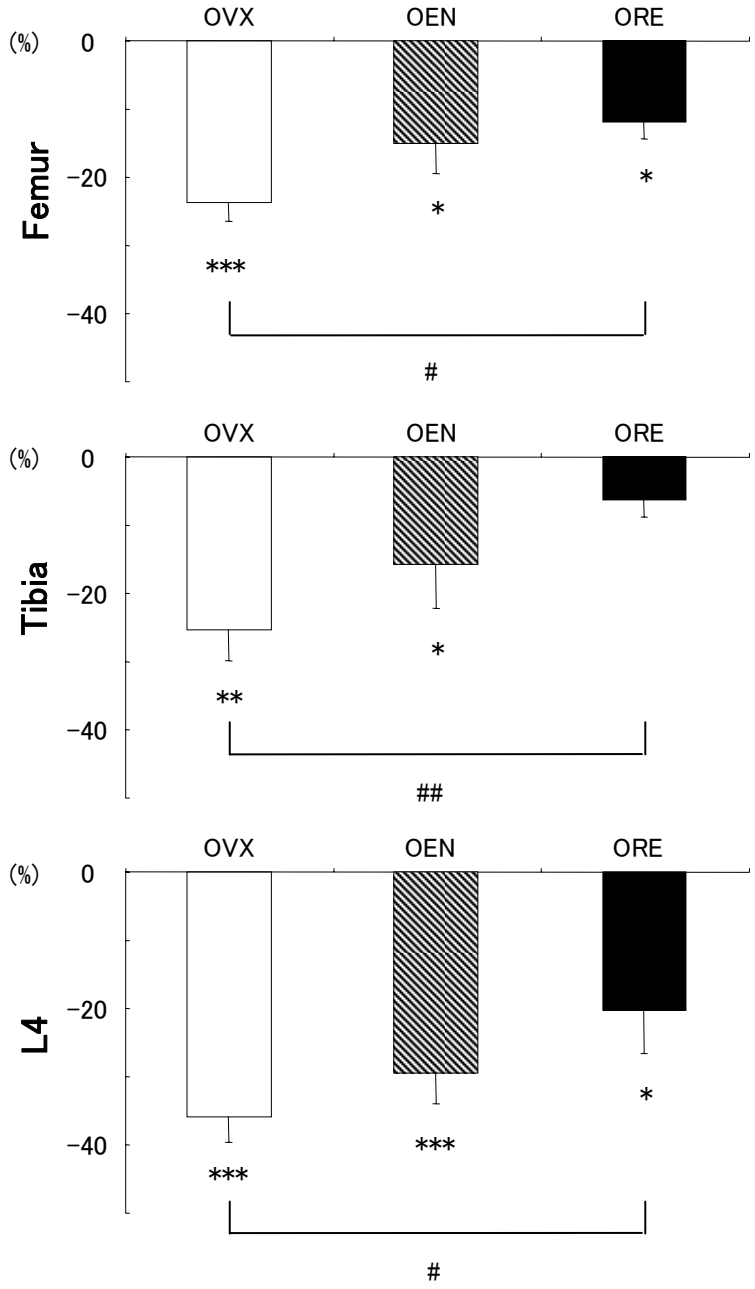

Fig. 2 Effects of endurance and resistance running exercise on bone mineral density measured by DEXA of femur, tibia, and L4 in rats shown comparison of ratio to Sham group (%). *, **, ***: $p<0.05$, 0.01, 0.001 vs Sham group.#, ##: $p<0.05$, 0.01 vs OVX. OVX; ovariectomized, OEN; endurance running exercise with OVX, ORE; resistance running exercise with OVX. OVX was performed at 12 weeks, and exercises treated from age of 14 to 18 weeks.

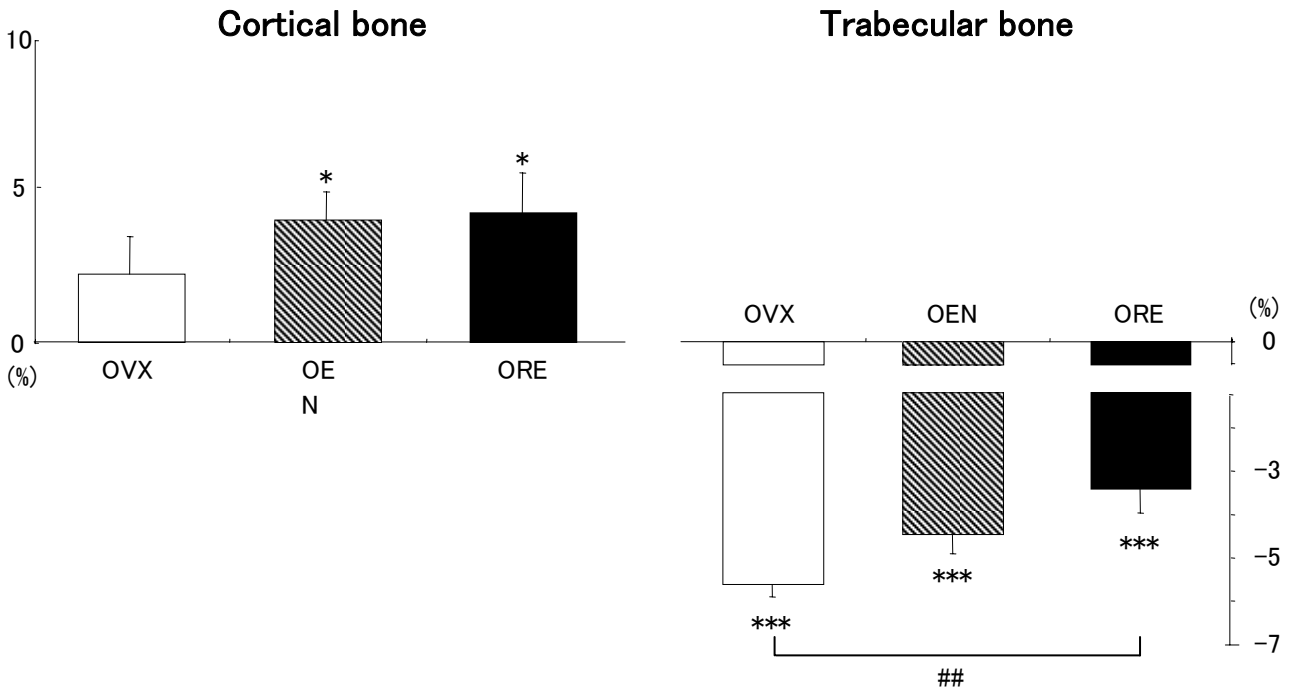

Fig. 3 Effects of endurance and resistance running exercise on cortical and trabecular bone mineral density of tibia measured by pQCT in rats shown comparison of ratio to Sham group (%). Values are Means±SE. *, ***: p<0.05,0.001 vs Sham group. ##: p<0.01 vs OVX. OVX; ovariectomized, OEN; endurance exercise with OVX, ORE; resistance exercise with OVX. OVX was performed at 12 weeks, and exercises treated from age of 14 to 18 weeks

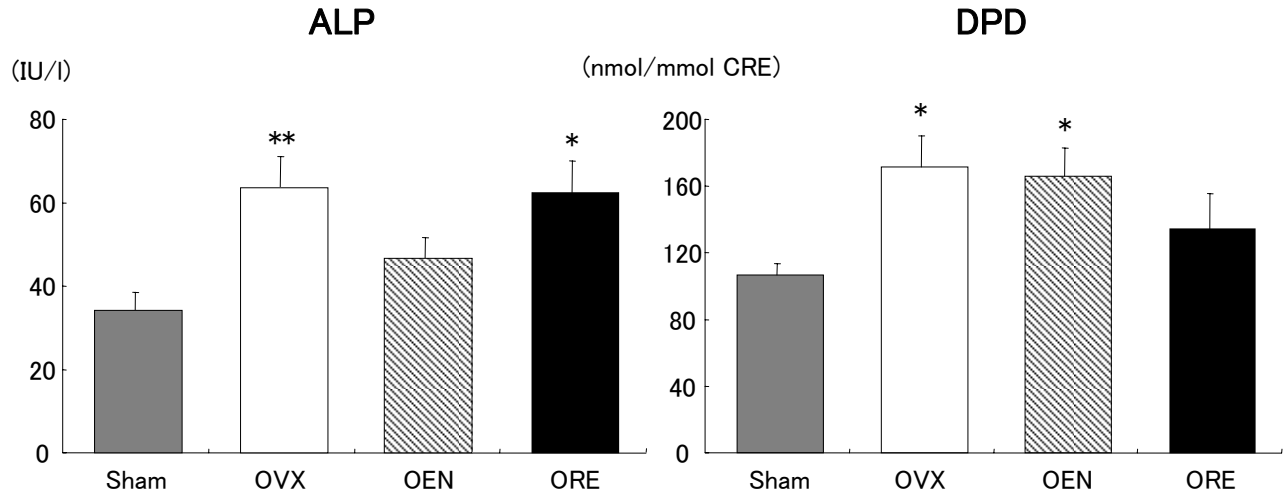

Fig. 4 Effects of ovariectomy (OVX), endurance and resistance running exercise on bone metabolic marker; serum ALP (IU/l) and urinary excretion of Deoxypyridinoline adjusted creatinine (DPD; nmol/mmol CRE) in rats. Sham; sham operated, OVX; ovariectomized, OEN; endurance running exercise with OVX, ORE; resistance running exercise with OVX, Values are mean±SE. *, ** : p<0.05, p<0.01 vs. Sham group.

Discussion

Sex hormone participates and keeps dynamic balance of bone metabolism in women and female animals. OVX rats were reported to induce the bone loss compared with sham rats (Kalu[13]1991).

In this study, BMD of femur, tibia and fourth lumbar vertebrae (L4) measured by DEXA and tibial trabecular BMD measured by pQCT were significantly decreased by OVX. Concerning bone metabolic markers; 24 hours urinary excretion of deoxypyridinoline adjusted creatinine as bone absorption marker in OVX were significantly higher than those of Sham. Thus, Estrogen deficiency accelerates bone resorption and results in rapid bone loss with high bone metabolic turnover.

Loading mechanical stresses as physical exercise are well established to inhibit bone loss caused by estrogen deficiency. Frost[8] (1992) has proposed a hypothesis to explain the effects of mechanical stress on the adaptation responses of bone. According to this hypothesis, repeated strong stimulus to a bone would restrain metabolic turnover and result in maintaining bone mass and consequently mechanical stimulus can counterbalance estrogen depletion and prevent postmenopausal bone loss through the inhibition of increased bone resorption. Physical exercise as give mechanical stimulus on bone has various types and forms with different effects.

In the present study we confirmed that the tibial trabecular BMD reduced in OVX rats to 56% of Sham operated ones, was significantly increased by resistance running exercise. There have been presented evidences in rats regularly running on a treadmill supporting Frost's proposal (Yeh[24] et al., 1993). There has been published observation suggesting resistance exercise training acted as dynamic stimulation to bones and operated effectively for increasing bone mass. BMD and bone mineral content (BMC) of body builders (Heinrich[10] et al., 1990), weight lifters (Bennell[4] et al., 1997) and power athletes (Snow-Harter[19] et al., 1992) have been indicated to be higher than those of distance runners and swimmers. These observations suggest that the efficacy of physical exercise for preventing bone loss could be varied with its types, and it is important how to give stimulus and gravity load on bones. In animal experiments, using normal conventional rats, various types and forms of exercises have been examined for the effects on increasing bone mass and density, such as jump training (Umemura[20] et al., 1995), low-intensity running combined with sudden impact-loading (Javinen[12] et al., 1998), squat training (Westerlind [22]et al., 1998), nonphysiological mechanical stimulation as low vibration of 50 Hz (Flieger[7] et al., 1998), frequently making rats to erect on bipedal stance for feeding (Yao[23] et al., 2000) and tower climbing exercise (Notomi[18] et al., 2001). These different types of exercise have been shown to increase bone mass by acting as a direct stimulus to working bones. There are several reports indicating that OVX rats were rather sensitive to loaded stimulus and gained greater suppressive effects of treadmill running on bone loss caused by estrogen deficiency (Flieger[7] et al., 1998).

Nakajima[17] et al. (2001) tried to develop and evaluate a new type of resistance exercise for increasing bone loss in OVX rats. We loaded a lead plate weighing 100g on the back of OVX rats, and made the weight bearing rats to intermittently walk at 20m/min interspersed with short rest on a uphill treadmill, in which the rats could receive more intense stimulus than those in previously published studies like the treadmill running exercise (Barengolts[2] et al., 1993). We termed this mode of workout as resistance exercise after the general definition that resistance-runs are repeated runs against an added resistance such as a hill, a weight carried, a drag which has to be pulled, or a soft, uneven

surface (Kent[14], 1994).

Our resistance exercise successfully increased the BMD and bone strength of femurs and L4 of OVX rats by approximately 6% and 7%, compared with sedentary counterparts. Barengolts[2] et al. (1993) reported endurance exercise (21m/min, 7 % inclination, 40min/day, 4days/week for 3 month) increased the ash weight of femurs and tibia. In this study, however, endurance training referred to Barengolts[2] et al. (1993) forced to rats for four weeks as same duration as resistance running group there was no suppressive effect on bone resorption caused by OVX. The effect may decrease in order to shorten training duration from three month to four weeks. From a view of bone metabolic marker, DPD in ORE was significantly lower as compared with Sham while there was no such tendency in OEN. This result suggested that resistance exercise inhibited bone resorption caused by OVX. Yeh[24] et al (1993) demonstrated that suppressive effects on bone resorption appeared after exercise 9 weeks although bone formation was not improved in 16 weeks in treadmill running (17m/min, 0% grade, 1hour/day, 5days/week, for 9weeks or 16weeks) in 14 month old rats. In this study endurance exercise had no effect on bone resorption. On the other, it was confirmed that resistance strengthened exercise suppressed OVX induced bone loss in four weeks as same duration as endurance exercise. It may be that the endurance exercise affected slowly on bone resorption because of difference of stimulation degree on bone as compared with resistance exercise.

When rats walk or run on in usual quadrupedal manners on a flat treadmill, additional gravity load to spine is very small. Iwamoto[11] et al. (1998) observed no significant difference in a maximum breaking force of the 5th lumbar vertebral body among rats loaded running at different speed and frequency of the exercise (12m/min 1h/day, 18m/min 1h/day and 12m/min 2h/day), and concluded that beneficial effects of treadmill running on bone strength came out only in weight-bearing bones. In the present study we loaded a weight of corresponding to about 40% of body weight on the back of exercising rats and drived those to run on a treadmill inclined up by 11%. The exercise trial we loaded exerted the small but significant effects on the L4 bone loss of OVX rats, and could be assessed to be a suitable way for transmitting a gravity stimulus to spine in rats.

It is known that ovariectomy promoted cancellous bone remodeling, and cancellous and cortical bone are lost in different degrees (Bagi[1] et al., 1997). In this study OVX reduced trabecular BMD not in cortical bone. Breen[5] et al., (1996) showed results that no significant difference in BMD between sham-operated and E2-treated OVX rats. Chen[6] et al., (1995) and Gasser[9] et al., (1995) demonstrated similar results. In this study cortical bone-rich tibial diaphysis BMD also did not affect by OVX and cancellous bone-rich tibial metaphysis BMD significantly decreased caused by OVX. These results suggested that six week OVX have no effect on cortical bone of eighteenth week old SD rat and OVX-induced BMD loss mainly came in cancellous bone for a while after OVX.

In conclusion, we evaluated the differential suppressive effects of endurance exercise and our new type of resistance exercise on the bone loss caused by OVX in rats. The results were obtained that the possibility that suitable resistance running exercise have more beneficial effects than endurance exercise on alleviating bone loss of ovariectomized rats because resistance strengthened running has stronger physical stimulation on bone as compared with endurance exercise and can inhibit bone loss in shorter duration, and loading back stimulates vertebrae and suppresses the bone loss. This preventing effects of bone loss may be caused by suppress

of bone resorption.

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