Dose-response Effects of Exercise Intensity on Bone in Ovariectomized Rats

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The purpose of this study was to investigate the effects of different intensities of resistance exercise training on established bone loss in ovariectomized (ovx-ed) rats by densitometry and histomorphometry. Thirty Female Wistar rats were ovx-ed or sham-operated (SHM) at 3 months of age and maintained untreated for 5 months after surgery to establish osteopenia. When they reached 8 months, the ovx-ed rats were divided into four groups in accordance with varying weights applied to a squat-training device: The weight classifications were 1) kept sedentary (OVX); 2) lifted 0 g (LOW); 3) 750 g (MID); and 1500 g (HIGH). The rats in the three training groups performed weight-lifting of 10 reps, performing 2 sets per day, 3 days a week for a ten week period. The Femora and tibiae were removed from each rat and were used for analyses. Ovx induced a significant loss of total BMC in all the bones tested. The ovx-induced femoral BMC loss was observed at all locations tested on the bone (proximal, shaft, and distal), and exercise-intensity dependent restoration was found at the proximal and the distal sites, but not at the shaft. In the tibia, ovx-induced significant bone loss occurred only at the proximal metaphyseal site. The training increased the tibial BMC of all sites in an exercise-intensity dependently, irrespective of the degree of ovx effect. At the tibial shaft, the training increased the cortical bone mass significantly above sham level by the bone apposition at the periosteum. At the proximal tibial metaphysis, exercise had no effect on the cancellous bone volume after ovx-induced bone loss. This finding suggests that the exercise induced bone increase in the ovx-ed rats was from cortical bone, not from cancellous bone, at least in the proximal tibia. These findings indicate that the weight-lifting exercise in rats reversed the ovx-induced bone loss in an exercise-intensity dependent and site-specific manner, even in established osteopenic skeleton 5 mon after ovx.

Keywords: postmenopausal osteoporosis, ovariectomy, mechanical load, weight-lifting

1. Introduction

Living in the aging society, the rapid bone loss after menopause (postmenopausal osteoporosis), which is observed among many middle-aged and older women, has garnered attention being seen as the underlying etiology of bed-ridden senior citizens these days. Physical exercise has been encouraged as one measure to cope with this problem, because it has become clear that the mechanical load on bones is the major influence in controlling osteogenic effects [Layne and Nelson (1999); Smith and Gilligan]
Training experiments have been frequently conducted to investigate the effectiveness and mechanics of the influence of exercise on osteoporosis through using ovariectomized (ovx-ed hereafter) rats as the postmenopausal osteoporosis model in studies up to the present. [Barengolts et al. (1993a, 1993b, 1994); Hart et al. (2001); Honda et al. (2001, 2003); Iwamoto et al. (1998); Kiuchi et al. (1997); Nordsletten et al. (1994); Peng et al. (1994, 1997)]. Results of these studies show that exercise can alleviate the bone loss by restraining the increased rate of bone resorption which starts immediately after menopause, which is effective in preventing osteopenia [Barengolts et al. (1994); Iwamoto et al. (1998); Kiuchi et al. (1997); Notomi et al. (2003)]. However, the effect of exercise as "treatment" when the increased rate of bone turnover and rapid bone loss immediately after ovx subsiding [Wronski et al. (1994)] has yet to be investigated.

The previous studies cited above examined bone response to different intensities of exercise but have yet to realize any irrefutable evidence [Barengolts et al. (1993a); Iwamoto et al. (1998); Katsuta et al. (1991); Peng et al. (1997); Wheeler et al. (1995)]. It is assumed that an increase in running speed is not directly reflected in the increase of mechanical load on the bone during treadmill running models that were applied in these studies. In order to investigate the relationship between exercise intensity and bone in detail, a resistant training model is considered to be suitable because it can exert a large mechanical load on bones during exercise and can offer a wide range of exercise intensity. Although weight-lifting [Tamaki et al. (1992, 1997)], jumping [Honda et al. (2001, 2003)] and tower climbing [Notomi et al. (2003)] have been contrived as the resistant training model of rats, a study has not been conducted yet to examine the relationship between exercise intensity and bone using these experimental models.

Therefore, the purpose of this study was to examine 1) whether physical exercise can restore the bone state, starting from 5 months after ovx at such a time when the bone mass decrease has reached the static state [Wronski et al. (1989)] and 2) whether there is a difference of effectiveness caused by the difference in exercise intensity, from the view points of in vivo & ex vivo densitometry and histomorphometry using the weight-lifting exercise model of rats.

2. Materials and Methods

2.1. Treatment of animals

Thirty female Wistar rats aged 11 weeks were purchased from Shimizu Experimental Supplies (Kyoto, Japan). They were allowed to acclimate the feeding condition with normal diet (Oriental MF: Ca 1.2%, P 0.9 %, and vitamin D 80 U/100g, Oriental Bio, Kyoto, Japan) and tap water ad libitum under a 12/12 hour light/dark cycle for 1 wk prior to the start of the experiment. At 12 weeks of age, animals were sham-operated or ovx-ed under pentobarbital anesthesia (12.5:2.5 mg/kg, respectively). Sham-operated rats kept sedentary throughout the experiment (group SHM). Established bone loss was induced by ovx followed by no-treatment for 20 weeks. Then ovx-ed rats were divided into four groups; a sedentary group (OVX group) and three resistance training groups.

The training group rats were trained with a modified weight-lifting exercise model first devised by Tamaki et al. (1992, 1997). While the original model used electronic shocks in the tail of rat as a stimulus to raise weights, the current study followed Oishi et al. (2004) and adopted the touch stimulus. When the rats became used to the weight-lifting exercise, the touch stimulus was decreased. The training rats were divided into three groups according to the weight attached to the weight-lifting device (group LOW, MID, and HIGH). The rats were trained one by one by the same trainer. Figure 1 shows the weight loaded to the squat training device. The LOW group lifted their own weight without a load placed on the device. All rats of the three training groups lifted 2 sets of weights in 10 daily repetitions, three days a week for ten weeks.

The experimental protocol was approved by The Animal Subjects Committee at Setsunan University.

2.2. Bone mineral analysis

Bone mineral content (BMC) measurements were done using dual-energy X-ray absorptiometry (DCS-600; Aloka, Tokyo, Japan) adapted to measurement in small animals. The longitudinal in vivo BMC data of the whole femur were measured at the beginning and the end of training period while under anesthesia comprising a compound liquid of ketamine and xylazine (12.5: 2.5 mg/kg). The ex vivo
total BMC (t-BMC) was measured at the femur, tibia and humerus which was removed after sacrifice at the end of the training period. The femur and tibia scan was divided into the proximal 1/3, the shaft 1/3, and distal 1/3 part.

2.3. Bone sampling

At the end of 10 weeks of programmed exercise, all rats were killed by aortic exsanguination under pentobarbital sodium anesthesia (0.1 ml/100 g body wt ip). The tibia and femur were fixed in the 75% ethanol solution after removing soft tissues, in order to be used in the BMC analysis and the bone histomorphometry.

2.4. Bone histomorphometry

Excised femur and tibia were embedded undecalcified in methyl methacrylate resin after Villanueva bone staining. The cross sections just proximal to the tibiofibular junction were cut with a Micro Cutter (MC-201; Maruto, Tokyo, Japan) and ground to a thickness of 50μm with a Speed Lap (ML-521D; Maruto, Tokyo, Japan). The longitudinal sections at the proximal tibia were cut with a microtome (Autocut/Jung 2055; Leica, Nussloch, Germany) at an indicated thickness of 5 μm. All sections were coverslipped with EUKIT for histomorphometry.

The digitizing morphometry system was applied for the histomorphometry. The system is composed of a fluorescence microscope (BX60; Olympus, Tokyo, Japan), a digitizing pad (UD-1212II; Wacom, Saitama, Japan) connected to the NEC computer and the semi-automatic image analyzing system (MGA-4300; System Supply, Nagano, Japan).

The cortical bone parameters were as follows; cortical bone area, marrow area, total (marrow + cortical) area, and moment of inertia. The cancellous bone measurements at the proximal tibial metaphysis were made at more than 1mm distances from the growth plate-metaphyseal junction to exclude the primary spongiosa. The cancellous bone parameters were as follows: BV/TV (%): bone volume/tissue volume corresponding to the part of cancellous space filled with trabeculae; Tb.Th (μm): mean thickness of the trabeculae; Tb.N (#/mm): mean number of the bone trabeculae; Tb.Sp (μm): trabecular separation that estimates the distance between two trabeculae.

2.5. Statistical analysis

All data were expressed as the mean ±SD. Fisher’s PLSD was used for the subtest after the analysis of variance to perform the intergroup trial. The significance level was set at $p < 0.05$. 

Figure 1  Training program
3. Results

The final weights of rats were as follows; SHM: 342.0 ± 24.8g, OVX: 376.3 ± 27.6g, LOW: 372.3 ± 25.6, MID: 351.0 ± 23.4g, High: 365.7 ± 11.1g. OVX and LOW showed significantly heavier weight than SHM \((p < 0.05)\). However, significant weight difference was not observed among the four groups of ovx-ed rats.

Figure 2 shows the longitudinal data of in vivo femoral t-BMC before and after the 10 weeks of resistance training. The femoral t-BMC decreased significantly during the period of 20 weeks after ovx (-18%). The recovery of femoral t-BMC, which is dependent to exercise intensity, was observed at the end of 10-week training period. BMCs of the excised femur and tibia (Figure 3) showed exercise-intensity dependent recovery after ovx.

When the three parts (proximal, shaft, and distal) of femur and tibia bones were compared, it was found that the response to ovx and exercise differed between the femur and tibia (Figure 4) The bone-loss in the femoral BMC induced by ovx was observed in all three regions and the recovery of BMC due to exercise was observed at the proximal and distal site, but not at the shaft. of BMC due to exercise was observed at proximal and distal locations. However, the loss of tibial BMC was observed only at the proximal site and the increase in BMC which is dependent to exercise intensity was seen irrespective of the ovx influence.

Figure shows the cross-sectional morphology at the tibial shaft. Similar to the results of the tibial shaft BMC (Figure 4), ovx did not affect the cortical bone area of the tibial shaft. Contrarily, the marrow area was significantly enlarged by ovx, indicating an ovx-induced facilitation of endosteal bone resorption. The exercise in the MID and HIGH groups prevented this progress. The tissue area, the cortical area, and the area moment of inertia did not change with ovx, but increased with training in an exercise-intensity dependent manner.

Figure 6 shows the cancellous bone structural indices at the proximal tibial metaphysis. The
ovx-induced decrease in BV/TV, Tb.N, and increase in Tb.Sp were not affected significantly by any intensity of resistance training. However, rats in MID and HIGH groups had significantly thicker trabecula than the SHM group.

4. Discussion

The findings of the present study suggest the possibility and limitation of physical exercise intervention into the bone loss induced by estrogen...
deprivation. This means that, the longitudinal data by in vivo DXA (Figure 2), ex vivo DXA (Figures 3 and 4) and cross-sectional data from the histomorphometry (Figures 5 and 6) strongly suggest that training can restore the bone volume of the femur and tibia depending on the intensity of exercise. It is also suggested that these positive impacts were site-specific, and any intensity of training could not restore the reduced bone by ovx at the proximal tibial metaphysis which has the high cancellous bone rate (Figure 6). This result differs from previous studies which loaded ovx rats with exercise in two ways:

1) the lower intensity training group showed higher effect in the previous studies to train ovx-ed rats with different intensity exercise [Barengolts et al. (1993a); Iwamoto et al. (1999); Peng et al. (1997)] and 2) the training on ovx-ed rats has positive influence in restoring the cancellous bone volume [Barengolts et al. (1994); Kiuchi et al. (1997); Notomi et al. (2003); Peng et al. (1992, 1997)].

Barengolts et al. (1993a) trained one-year-old rats three months after ovx. Their three months of treadmill running increased the tibial ash weight more in lower speed group (12 m/min) than in higher speed group (21 m/min). In addition, Peng et al. (1997) trained 13-week-old rats one week after ovx and found the more pronounced increase of bone parameters (the maximal load of the femoral neck, and the cancellous bone volume at the distal femoral metaphysis) in the 12m/min trained rats than in the 18m/min trained. Iwamoto et al. (1998) also loaded 3-month treadmill running on nine months old rats of three months after ovx at the same speed as the running of Peng et al. (1994) and observed the high tibial density and wet weight in the 12 m/min running group. The reason why the higher intensity exercise group had provided less skeletal response in these previous studies may be due to the lower training frequency of 1 day/week for high intensity exercise group [Barengolts et al. (1993a)], and the lower body mass induced by the enhancement in energy consumption for the high intensity exercise [Barengolts et al. (1993a); Peng et al. (1997)]. When examining the bone response against different exercise intensities, the study should ensure that other conditions (frequency and duration) and mechanical environment (body mass) are standardized. In this respect, although the all three exercise groups had lower average weight than the ovx-ed group, the difference was not significant in the present study. Also, all the exercise conditions except exercise intensity were equal in the all three exercise groups. Therefore, it is assumed that the difference of bone response among the three exercise groups in the present study is induced by the difference in the exercise-intensity itself.

Figure 6 Cross-sectional morphology tibial shaft
Data are mean ± SD. Significant differences from Shm, Ovx, Low, Mid, or High are depicted as "s," "o," "l," "m," or "h," respectively.
According to the histomorphometry at the proximal tibial metaphysis, it was demonstrated that the reduced cancellous bone due to ovx could not be recovered by any training with any load intensity in this study (Figure 6). Findings suggest that the progress of the loss of trabecular over a long period of five months after ovx caused the result. The study of Notomi et al. (2003) which showed the complete recovery to the Sham level, commenced training three months after the ovx, two months earlier than the present study. Barengolts et al. (1993a, 1993b, 1994) obtained the results that the degree of training-induced recovery from the bone loss induced by ovx is affected by the length of period, so the effectiveness of restraining bone reduction is higher when the exercise started immediately after ovx. The decrease in trabecular connectedness indicated by the increase in the number of trabeculars (Tb.N) means the progress of the loss of trabecular, suggesting trabecular could not exist at the spot after the progressed bone-loss. In other words, thinned trabecular can be thickened, while the lost bone cannot be restored. This seems to have restricted the cancellous bone recovery, which meant that any training with any exercise intensity could not exert an effect on the parts of higher cancellous bone ratio (proximal tibial metaphysis).

Although an exercise effect was not observed in the cancellous bone mass at the proximal tibial metaphysis as quantified in the histomorphometry (Figure 6), it is interesting that proximal tibial BMC by DXA showed the bone volume recovery depending on exercise intensity (Figure 4). The result indicates that the bone responded to the exercise at the cortical bone site and that even the intense mechanical load of the present study could not affect the main factor with the ovx itself induced. It means that the estrogen secretion itself, which is the main factor of the bone reduction, is not affected by exercise. Because the exercise reversed the ovx-induced bone loss in an exercise-intensity dependent and site-specific manner even in such an estrogen-depleted state, physical exercise is assumed to exert the effect through the local factor due to the increased mechanical load on the bone without the change in endocrine system such as estrogen secretion. Our view seems to be supported by the previous study [Shimegi et al. (1994)] in which the negative correlation between the years after menopause and bone mineral content of the distal radius was observed, even in the postmenopausal exerciser (volleyball or jogging) who had significantly higher vertebral bone mineral content.

Frost (1987a, 1987b) modeled the relationship between the bone tissue strain due to mechanical stress and the bone metabolism control as "mechanoestat theory". The basic concept underpinning this theory is that the degree of remodeling and remodeling changes depending on the strain occurring at the bone. It is necessary for the strain to exceed the set point in order to activate remodeling and to restrict remodeling. The set point is called "minimum effective strain: MES". The activation of remodeling is induced by strains which exceed the minimum effective strain of modeling (MESm). In contrast, remodeling is restricted by strains which exceed the minimum effective strain of remodeling (MESr) and it is activated when the strain becomes under MESr. The positive effect for ovx-ed and sham/intact rats are identified differently: attenuation of bone loss by suppressing the increased remodeling for ovx-ed rats, and bone gain by accelerating modeling for sham/intact rats. Previous studies often reported that physical exercise is effective only for ovx-ed rats but ineffective for sham/intact rats [Barengolts et al. (1993a, 1993b); Kiuchi et al. (1997); Peng et al. (1997)]. This may mean that the treadmill running induces the mechanical strain of "conservation mode" (MESr<strain <MESm), so that the exercise of the intensity may restrict an increased remodeling but cannot accelerate modeling. Thus, the bone gain and the restoration of ovx-induced bone loss which is dependent to exercise intensity in the femur and tibia (Figures 2-4) in the present study indicates that weight-lifting exercise of rats has the mechanical load over the MESm level which promotes the modeling at the periostium. Estrogen is known to increase the sensitivity of bone to mechanical loads [Lee et al. (2004)]. Based on this, ovx or menopause would decrease the sensitivity of bone to mechanical loads (raising the set point). Therefore, the estrogen deficient bone cannot detect smaller load intensities as osteogenic stimulus. In addition it was noticed that a training effect was observed only for the premenopausal women when pre- and postmenopausal women were similarly loaded with the same exercise (vertical jump) [Bassey et al. (1998)]. Additionally, the report of Honda et al. (2003) where no difference was observed between
ovx-ed rats and intact rats suggests that the jump training of rats exceeded MESm. The ground reaction force during weight-lifting exercise was about four times greater than the body mass in the LOW group, about seven times of the MID group and eight times of the HIGH group (in our laboratory data), while about five times during jumping in the study of Honda et al. (2003). Therefore it is assumed that the weight-lifting exercise had the mechanical load adequate enough to exceed MESm.

The condition of bone restoration through resistance training after ovx differed between the femur and tibia in the present study (Figure 3) and the responses differed by the site (proximal, distal and shaft) in the same bone (Figure 4). That is, the significant BMC decrease due to ovx was observed in femur bones at each site, while the BMC increase due to training was observed at the proximal and distal parts. Honda et al. (2003) loaded two-month jumping training on one-year rats three months after ovx and gained the same result. In contrast, the significant BMC decrease due to ovx was observed in the tibia only at the proximal part while the BMC increase depending on exercise intensity of the training was observed at each site irrespective of ovx (Figure 4). It is suggested that the difference in response against exercise between the femur and tibia is caused by the fact that the threshold differs by the part of the same bone responding to the mechanical load [Hsieh et al. (2001)], as well as the fact that the way to burden the mechanical load (strength and direction) differs by anatomic position of the bone during exercise.

In the exercise training experiments in ovx-ed rats so far, the unattended period after ovx was at most three months [Barengolts et al. (1993a); Honda et al. (2003); Iwamoto et al. (1998); Notomi et al. (2003)]. The present study adopted as an unattended period a time span of five months after ovx for the first time. Consequently the bone-loss progressed so far that the exercise effect and limitations were clearly observed as "the treatment" after the progressed osteoporosis. In addition, previous studies adopted treadmill running models for the training experiments in most cases, and a few of them adopted resistance training models just to examine the bone response in only one exercise intensity [Honda et al. (2001, 2003); Notomi et al. (2003)]. This study widened the parameters with a specified wide range of "mechanical exercise intensity", examined the dose-response and elucidated the bone adaptation depending on exercise intensity. The exercise intensity in the present study is considered impossible to achieve in the mechanical environment setting in treadmill running. Some future studies are anticipated on the relationship between exercise intensity and bone using this training model applied here.

In conclusion, the present study indicated that the resistance training in rats reversed the ovx-induced bone loss in an exercise-intensity dependent and site-specific manner mainly by the cortical bone response, even in established osteopenic skeleton 5 month after ovx. However any intensity of resistance training could not recover the cancellous bone loss after ovx. Therefore, in order to improve the effectiveness of exercise intervention as a treatment of postmenopausal osteoporosis, the following two points should be considered; 1) exercise intervention should be started as early as possible to keep the bone trabecular connection in order to stop the loss of cancellous bone due to the bone resorption progress immediately after ovx or menopause; 2) strong mechanical loads such as resistance training should be actively given to restore the bone mass when the adequate bone volume remains at the time of ovx or menopause.

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