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Mechanical Stimulation in the Form of Vibration Prevents Postmenopausal Bone Loss in Ovariectomized Rats

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Abstract. Physical exercise is recommended for the prevention and treatment of osteoporosis. However, its exact role and effectiveness in adulthood is unclear. While vigorous exercise of long duration enhances bone density, few adult individuals comply with such training programs. The present study evaluates the influence of nonphysiological mechanical stimulation, in the form of low intensity vibration (frequency: 50 Hz, acceleration: 2 g, 30 min/day for 5 days/week), on the prevention of bone loss in an animal model of postmenopausal osteoporosis. In the ovariectomized groups of rats a statistically significant ($p < 0.05$) decrease of bone density (femur and tibia) was recorded at 5 weeks postovariectomy. This effect was maintained for the 12 week duration of the study. Vibration prevented early bone loss after ovariectomy. Vibrated ovariectomized rats showed statistically significantly higher ($p < 0.05$) BMD values compared to those of their ovariectomized controls at 5 weeks. Vibration did not influence the bone density of the SHAM-operated rats. Although vibration increased ultimate strength (fracture load of the rat femur) in the ovariectomized rats, this finding was not statistically significant. Our data indicate that this method of safe and easily applicable vibration, in the form of a vibrating platform, is effective in preventing early postovariectomy bone loss in an animal model.

Key words: Vibration — Osteoporosis — Bone mineral density — Strength.

Postmenopausal estrogen deficiency leads to accelerated bone turnover and bone loss in humans (1, 2). Physiological mechanical stimulation in the form of increased physical activity and systematic body exercise, after the menopause, is considered to decrease bone loss and even to cause an increase in bone mass [3, 4]. However, data deriving from cross-sectional and longitudinal studies indicate that although exercise is very effective in developing bone mass in adolescence, it may have less effect in adults and in the elderly [5, 6]. Only vigorous exercise of a certain type and of long duration, with which few individuals easily comply, results in significant BMD changes, and moderate exercise regimens cannot compensate for early postmenopausal estrogen and bone density lost [5, 6].

The influence of passive exposure to nonphysiological

mechanical stimulation has not been thoroughly investigated. Vibration is a kind of nonphysiological mechanical stimulation, and we are currently investigating its effects on normal and pathological bone. It has the advantage of easy application, even on sedentary individuals, with user-friendly, durable, and low-cost devices.

We present the result of an experimental study evaluating the influence of vibration on bone mineral density and bone mechanical properties in a model of ovariectomized rats [7].

Materials and Methods

Animals

In this study thirty-two adult (12-week-old) female Wistar rats (7 weighing approximately 200 g) were used. A special effort was made to avoid significant genetic and anatomical variations among the animals. The following process was used: female siblings from the same parents were evenly allocated to a given group and to the corresponding control group. The animals were ovariectomized or sham-operated under intraperitoneal anesthesia (ketamine, xylazine). They were then randomly allocated into four groups (8 rats each): 1) sham-operated control (SHAM-C), 2) sham-operated vibrated (SHAM-VIBR), 3) ovariectomized control (OVX-C), and 4) ovariectomized vibrated (OVX-VIBR). All animals were kept under the same conditions (climate and cages) having unlimited access to standardized food and water. The study lasted 12 weeks. The rats were killed with intramuscular injections of high doses of ketamine and xylazine. The body-weight of all rats was recorded at the following time points: 0, 5, 8, and 12 weeks.

Vibration

Starting from the third postovariectomy day, specially designed vibrating platforms (FIG. 1) were placed in the cages of groups OVX-VIBR and SHAM-VIBR and the animals of these groups followed a 12-week vibrating program (20 mm/day for 5 days/week). This platform was set to provide a vertical acceleration of 2 g ($a = 9.8 \text{ m/sec}^2$) and a frequency of 50 Hertz.

Bone Mineral Studies

Bone mineral studies were performed on all groups, at the initiation of the study and at 5, 8, and 12 weeks postoperatively. All rats were placed on a special holder to keep them in position for DEXA measurements. A LUNAR DPX-L (Lunar Radiarion Co, Madison, WI) device and a small animal software program with an appendicular mode scan were used. BMD measurements [8, 9] were obtained and analyzed in four different anatomical regions of the

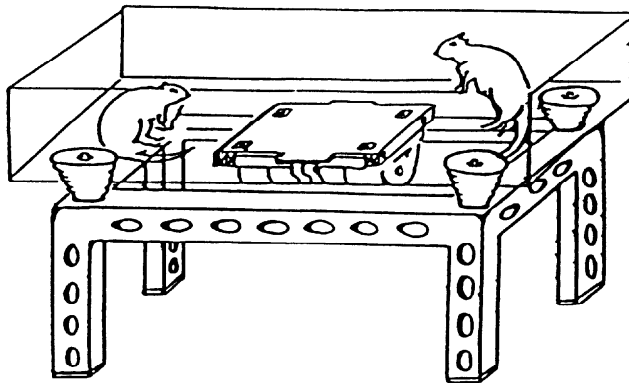


Fig. 1. Line drawing of the vibrating platform. An electric micro-vibrator (Micro 20TR, $N = 22$ Watt, $f = 50$ Hertz) is fixed under a metallic platform (45 cm/35 cm) over which a plastic cage of the same size is attached. The platform is lying on a metallic frame with 4 elastic vibrating cylinder placed at the 4 corners

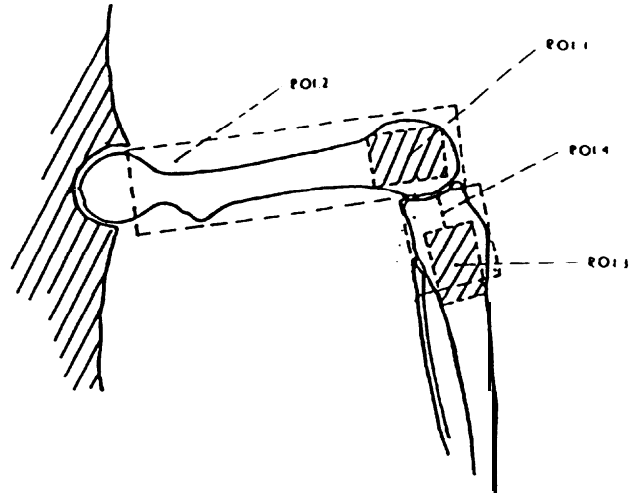


Fig. 2. Bone mineral density measurements in the rat femur and tibia (4 areas of interest-ROI's), following the placement of the animals on a special holder.

terest (ROI's) (Fig. 2). ROI 1 represented the femoral condyles (mostly trabecular bone), ROI 2 the whole femur (combination of cortical and trabecular bone), ROI 3 the proximal metaphyseal tibia area 2.5 mm distal to the Joint line (area of the secondary spongiosa) and the ROI 4 the proximal epiphyseal and metaphyseal tibial area (epiphysis and primary spongiosa). All DEXA scans were performed twice, after repositioning of the animal, and the average BMD was recorded for every ROI

The average coefficients of variation of the replicate measurements over the experimental period were ROI 1 = 1.58%, ROI 2 = 2.37%, ROI 3 = 2.45% and ROI 4 = 2.61%. Short-term precision, estimated within 24 hours on 10 animals at the beginning of the study, in our Laboratory was found to be 0.92% in ROI 1, 0.99% in ROI 2, 1.27% in ROI 3 and 1.35% in ROI 4. Finally, the stability of the measurement was controlled by scanning a phantom of known BMD every day

Mechanical Testing

The mechanical properties of the rat femur and tibia were studied using a Karl-Frank computerized testing machine. All specimens were kept wet in normal saline after harvesting, with the tests being performed within 20 minutes. A three point bending test (until failure) was used, applying a low strain rate of 0.0% at the site of the central elliptical cross section of the rat femur and at the triangular section of the rat tibia (10). Load-Deflexion curves were taken and the structural bone mechanical parameters of ultimate strength (fracture load N), Stiffness (N/mm), and toughness (N · mm²) were estimated from these (10)

Geometric Properties

After mechanical testing, the geometric properties of the femoral bone specimens (length, long and short external and internal diameters of the central elliptical cross section of the bone) were macroscopically recorded using a digitized Vernier calliper. Using the above cross sectional dimensions, the Inner (representative of the endosteal surface) and outer (representative of the periosteal surface) cross sectional areas were calculated

Statistical Analysis

Data are presented as means \pm SEM. Analysis of variance, with Scheffe's multiple comparison test was used to assess statistical

differences between the groups. A P value at the level 0.05 was considered significant.

Results

Changes in Body-Weight values (means \pm SEM) of the animals throughout the experiment are shown in Table 1. No differences between groups were recorded at the beginning of the study. A statistically significant increase of BW in the OVX groups compared to those which were SHAM operated was observed at 5 ($P < 0.05$), 8 ($P < 0.05$) and 12 ($P < 0.05$) weeks postovariectomy (Table 1). Vibration caused a nonsignificant decrease of BW at 8 and 12 weeks. The femurs of the ovariectomized group were statistically significantly longer ($P < 0.05$) than those of the SHAM operated groups (Table 2). Moreover the central inner elliptical cross-sectional area of the femurs in the ovariectomized groups was statistically significantly larger than those of the SHAM operated groups (Table 2).

BMD values in the 4 ROI's (means, \pm SEM) are shown in Figures 3-4. No differences between groups in the 4 ROI's were recorded at the beginning of the study. BMD in the OVX rats showed a statistically significant ($P < 0.05$) decrease at 5 weeks compared to that of the SHAM-operated groups. These differences remained significant ($P < 0.05$) throughout the duration of the experiment (Figs 3-6).

Vibration was found to preserve bone mass (BMD) at 5 weeks postovariectomy (Figs. 3-6). A statistically significant difference in BMD was observed when the groups OVX-VIBR and OVX-C were compared at 5 weeks ($P < 0.05$) (ROI 1 = 17%, ROI 2 = 13.8%, and ROI 3 = 14.1%) (Figs. 3-5). Although nonsignificant, these differences between OVX-VIBR and OVX-C groups were maintained at 8 and 12 weeks. A nonsignificant BMD increase from baseline was observed in the OVX-VIBR group at 5 weeks.

It must be stressed that vibration did not cause any BMD improvement in the SHAM-operated animals (SHAM-VIBR compared to the SHAM-C group).

Although the ultimate strength of the femur and

Table 1. Animals BW during the experiment

Group	N	BW-0w	BW-5w	BW-8w	BW-12w
SHAM-C	8	210.0 ± 4.4	228.8 ± 3.6	250.0 ± 5.8	258.3 ± 6.0
SHAM-VIBR	8	203.8 ± 5.4	231.9 ± 3.7	237.5 ± 4.3	245.0 ± 3.5
OVX-C	8	201.3 ± 4.4	258.1 ± 5.3 ^b	282.5 ± 7.5 ^b	307.5 ± 9.5b
OVX-VIBR	8	200.0 ± 3.0	253.8 ± 6.8b	277.5 ± 1.4^b	282.5 ± 2.5^c

. BW values expressed as mean ± SEM.

^b Statistically different (P < 0.05) from SHAM-C and SHAM-VIBR.

^c Statistically different (P < 0.05) from SHAM-VIBR.

Table 2. Rat femoral bone geometric properties 12w

Groups	N	Length (mm)	Internal cross-sectional area (mm ²)
SHAM-C	8	32.23 ± 0.4	11.68 ± 0.03
SHAM-VIBR	8	32.48 ± 0.23	11.90 ± 0.11
OVX-c	8	33.68 ± 0.24 ^a	12.68 ± 0.26 ^b
OVX-VIBR	8	33.65 ± 0.21	12.92 ± 0.12 ^b

^a Geometric properties values expressed as mean ± SEM.

^b Statistically different (P < 0.05) from SHAM-C and SHAM-VIBR.

Table 3. Rat bone ultimate strength^c 12w

Groups	N	Femur (N)	Tibia (N)
SHAM-C	8	114.05 ± 8.25	89.20 ± 6.29
SHAM-VIBR	8	121.65 ± 3.13	94.30 ± 1.72
OVX-C	8	111.75 ± 5.08	78.80 ± 5.64
OVX-VIBR	8	123.50 ± 1.54	81.60 ± 4.84

^c Ultimate strength values expressed as mean ± SEM.

No statistically significant differences were found

showed a tendency to be greater in the vibrated groups compared to that of the non-vibrated. this finding was not statistically significant (Table 3).

Discussion

Osteoporosis has become a socio-economic problem, affecting the aging population of our planet. Prevention, early detection of patients with high bone turnover, management of secondary osteoporosis, appropriate drug therapy, prevention of falls and orthopedic management of osteoporotic fractures are the cornerstones of the modern management of osteoporotic patients.

In recent years, Frost has developed a hypothesis to explain the effects of mechanical loading on adaptation responses of bone [11-13]. According to this hypothesis, the application of higher strains to the bone suppresses remodeling and conserves bone. As a consequence, mechanical stimulation can counterbalance estrogen depletion and prevent postmenopausal bone loss through the inhibition of increased bone turnover [13]. Although body exercise (physiological mechanical stimulation) is considered effective for the management of postmenopausal osteoporosis, as a complementary treatment to drug therapy, few patients comply with these long, rigorous and systematic programs (5, 6).

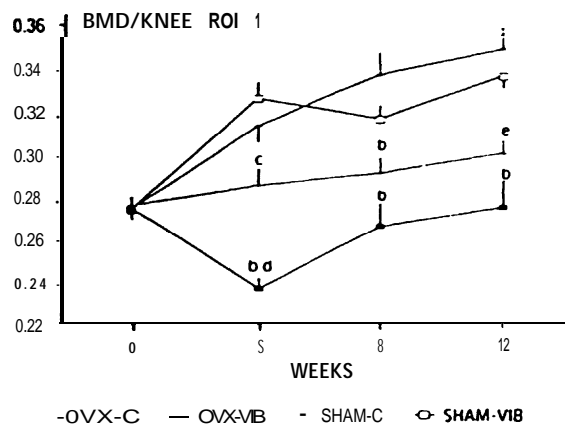


Fig. 3. BMD changes throughout the experiment in ROI 1 (rat femoral condyles). BMD values expressed as Mean ± SEM. N = 8. ^a Significantly different (P < 0.05) from SHAM-C and SHAM-VIBR. ^b Significantly different (P < 0.05) from SHAM-WBR. ^c Significantly different (P < 0.05) from OVX-ViBR. ^d Significantly different (P < 0.05) from SHAM-C.

The influence of dynamic (nonphysiological) mechanical stimulation has been investigated in traumatic bone models with the application of external fixators in avian and turkey bones [14, 15], or in models of ulna osteotomies [16, 17]. It has been found that physiological levels of strain imposed with an abnormal strain distribution can prevent intense remodeling and produce an osteogenic stimulus that is capable of increasing bone mass.

In this study, we evaluate the influence of nonphysiological mechanical stimulation on an experimental model of postmenopausal osteoporosis [7]. Vibration has been chosen as a form of nonphysiological mechanical stimulation because its hypothetical beneficial effect can be easily applied to humans with the use of simple, inexpensive devices such as vibrating platforms, without the need for effort on the part of the patient. Vibration effects on normal bone metabolism [18-20] and on fracture healing [21] have already been described. Surprisingly, there is only one report which evaluates the positive effect of vibration on the abnormal bone metabolism of disuse osteoporosis in paraplegic patients [22]. The potential harmful side effects of vibration on tissues other than bone (such as muscles, nerves and vertebral disks), especially if resonant frequencies of high intensity and long exposure time are used, may explain this [23-25]. For the needs of our study, safe frequency and low intensity vibration parameters [19, 25] of short daily duration were chosen with the aim of avoiding side effects [26-28] and of achieving, at the same time, the appropriate bone remodeling minimal effective strain values [13]. These

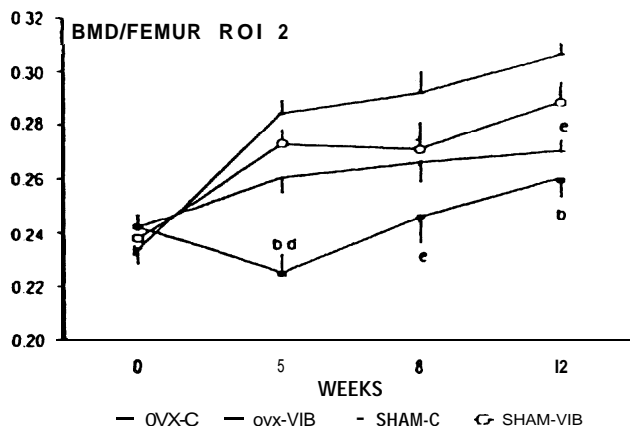


Fig. 4. BMD changes through the experiment in ROI 2 (whole femoral bone). BMD values expressed as Mean \pm SEM. N = 8. Significantly different ($P < 0.05$) from SHAM-C and SHAM-VIB. Significantly different ($P < 0.05$) from SHAM-VIB. Significantly different ($P < 0.05$) from OVX-VIB. Significantly different ($P < 0.05$) from SHAM-C

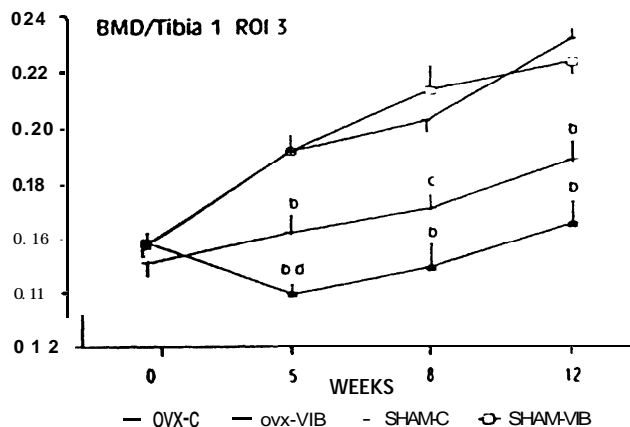


Fig. 5. BMD changes throughout the experiment in ROI 3 (ribial metaphysis). BMD values expressed as Mean \pm SEM. N = 8. Significantly different ($P < 0.05$) from SHAM-C and SHAM-VIB. Significantly different ($P < 0.03$) from SHAM-VIB. Significantly different ($P < 0.05$) from OVX-VIB. Significantly different ($P < 0.05$) from SHAM-C

vibration parameters. were effectively applied. through a specially designed platform. on a well established animal model of postmenopausal osteoporosis [7]

The results of the present study confirm the decrease of BMD in ovariectomized rats (7). It is known that ovariectomy in rats stimulates bone remodeling and bone loss [7]. An early post-ovariectomy increased bone remodeling rate that decreases later has been reponed [29. 30]. In our study. bone loss was more pronounced in the early post-ovariectomy period (5 weeks) and in the metaphyseal areas of bone (ROI 1 = 13.8% and ROI 3 = 12.1%-change from the base line). The decrease of BMD was less severe when the whole bone and epiphyseal-metaphyseal areas were considered (ROI 2 = 6.9% and ROI 4 = 7.6%). vibration prevented this early post-ovariectomy bone loss (5 weeks). Although the difference was not statistically significant. vibration also helped ovariectomized rats maintain higher BMD throughout the duration of the experiment. It must be

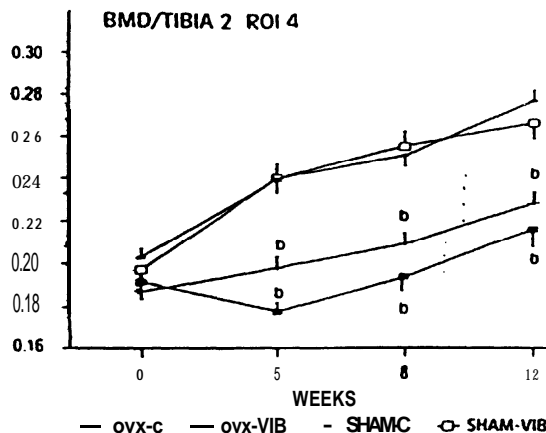


Fig. 6. BMD changes throughout the experiment in ROI 4 (tibial epiphysis and metaphysis). BMD values expressed as Mean \pm SEM. N = 8. Significantly different ($P < 0.03$) from SHAM-C and SHAM-VIB.

stressed that in all groups (even in the ovariectomized animals after the 5th week) a constant increase of bone mass was observed. This finding is explained by the fact that, although rats are considered as adults after the third month of their life, they keep growing slowly thereafter with bone modeling being active [7. 31. 32].

The question is how to explain the effects of vibration. The rate of remodeling activity is very low in rats, but it is activated after ovariectomy. resulting in a negative bone balance [7]. Moreover, ovariectomy increases both the length of the long bones by stimulation of longitudinal growth and the inner cross sectional area (cortex) by stimulation of endosteal bone resorption [32]. These findings were confirmed by our study. The efficacy of agents and exercise regimens in the immediate post-ovariectomy period can be evaluated in terms of their ability to prevent bone loss through inhibition of bone turnover (7). Thus. in seems that vibration exhibits a suppressant effect on increased bone turnover. From the mechanical point of view. the lack of estrogen, which raises the remodeling set points. causes modeling to stop increasing bone mass and puts remodeling into its disuse mode decreasing bone mass. Vibration, with the application of increased stresses on the bone. probably switches the remodeling conservation mode ON and thus preserves bone (33). On the other hand. in our study. vibration failed to increase BMD in the ovariectomized and non ovariectomized rats. An increase of BMD would only appear if bone modeling was stimulated by the application of vibration. Modeling is suppressed during the early post-ovariectomy period. when the osteopenia is developing [7] and for this reason it is not possible for vibration to increase BMD in ovariectomized rats. Moreover. in this study. vibration did not cause any significant change of BMD in the non ovariectomized rats, an indication that it does not have an effect on bone modeling.

Although nonstatistically significant. vibration showed a tendency to improve the mechanical properties of conical bone. It seems that changes of bone strength may take time to develop (bone remodeling-adaptation) and always follow bone mineral density alterations. Since the improvement of bone strength is more important than positive effects on bone mineral density. further long-term studies evaluating the effects of vibration on bone are necessary.

It must be emphasized that vibration affected mamma-

lian bone tissue (rat bone) in a similar way to the effect of dynamic loading on avian and turkey bone tissue. The fact that non physiological mechanical stimulation causes analogous bone effects in different species allows for the results to be extrapolated to humans. Such vibration effects could prove useful in the management of human conditions of increased bone turnover and bone loss (e.g., postmenopausal and disuse osteoporosis). The use of low intensity vibration regimens will avoid the potentially harmful effects of this form of nonphysiological mechanical stimulation. Further studies should be conducted to confirm and evaluate the efficacy of different vibration regimens on both cortical and trabecular bone, and to develop durable, easy to use and cost effective vibrating platforms.

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