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Original Article

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A novel method to "exercise" rats: making rats rise to erect bipedal stance for feeding - raised cage model

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Abstract

We employed a novel method to exercise rats: making them rise to bipedal stance for feeding using raised cages. We studied its effects on the skeletons of 6 and 10-month-old intact or orchidectomized (ORX) rats. Body and hindlimb muscle weights, tibial BMC and periosteal cortical bone formation increased after housing in raised cages, but more so in 6-month-old animals than in 10-month-old ones. In 6-month-old orchidectomized rats, raised cages partially prevented ORX-induced bone loss by stimulating periosteal cortical bone (TX) formation and decreased bone resorption next to marrow. In 10-month-old male orchidectomized rats, raised cages also decreased the endosteal and trabecular bone resorption, but not enough to prevent completely ORX-induced net bone losses. Because the osteogenic effects of raised cages alone were only partial, we also studied the interaction between raised cage and prostaglandin E_2 (PGE_2) in 10-month-old retired female breeders. When treated with combined raised cage and PGE_2, both cortical (TX) and trabecular bone mass of the proximal tibial metaphysis and lumbar vertebral body increased over either raised cages or PGE_2 treatment alone, that was accompanied by dramatic increased bone formation at periosteal and endosteal surfaces. Thus making rats rise to erect bipedal stance for feeding helps to prevent bone loss after orchidectomy; it amplifies the anabolic effects of PGE_2, and it provides an inexpensive, non-invasive and reliable way to increase mechanical loading of certain bones of the rat skeleton.

Keywords: Exercise, Rats, Orchidectomy, Histomorphometry, Cancellous, Cortical Bone

Introduction

The current methods to exercise rats include treadmill running[1-3], jumping[4], swimming[5], climbing ladders[6], running wheels in cages[7,8,9] and overloading one limb by immobilizing the other[10-12]. Generally, these studies are labor intensive and time consuming to train and to exercise the animals. For example, the treadmill running and swimming studies took from 30 minutes to one hour per rat/per day, jumping took about 5 minutes per rat/per day and climbing a ladder took about 15 minutes per rat/per day. Having rats run wheels in cages consumes less labor but requires a lot of space: mostly one rat per cage. In order to minimize such time and labor problems we raised the height of the cages so the rats had to stand on their hind limbs to obtain food and water. Minimal technician time and labor was involved in this way of exercising rats. It requires no surgical or other form of intervention, and no regional acceleratory phenomenon occurs. There is no major change of the normal environment. It is less stressful than enforced treadmill or wheel running. The model also mimics the upright human posture.

These studies concerned the effects of this exercise model on the skeleton and muscle of different age/gender rats and its interaction with hormone-deficiency (androgen) or the use of an anabolic agent (PGE_2).

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Material and methods

Study 1: to test the effects on skeleton and muscle by making rats rise to bipedal stance for feeding of 6-month-old orchidectomized rats.

Six-month-old male Sprague-Dawley (SD) rats were divided randomly into the following 5 body-weight-matched groups with 6 rats per group:

1. Baseline control (Baseline)
2. Sham-orchidectomized plus housing in normal height

cages (58 cm x 36 cm x 20 cm; Sham + NC)
3. Bilaterally orchidectomized plus housing in normal height cages (ORX + NC)
4. Sham-orchidectomized plus housing in raised cages (58 cm x 36 cm x 28-35.5 cm; Sham + RC)
5. Orchidectomized plus housing in raised cages (ORX + RC)

The study lasted for 12 weeks.

Study 2: to test the effects on skeleton and muscle by making rats rise to bipedal stance for feeding in 10-month-old orchidectomized rats.

The experimental design was the same as study 1 except 10-month-old male SD rats were used, i.e., they were between 10 to 13 months of age during the study.

Study 3: to study the effects of raised cages and an anabolic agent, PGE_2, on female retired breeders (pilot study).

Five body-weight-matched groups of 10-month-old female retired Sprague-Dawley rats with 3 rats per group were employed:

1. Baseline control (Baseline)
2. Aging controls plus housing in normal height cages (NC)
3. Prostaglandin E_2 (PGE_2) 1mg/kg/d plus housing in normal height cages (NC)
4. Aging controls plus housing in raised cages (RC)
5. PGE_2 1mg/kg/d plus housing in raised cages (PGE_2 + RC)

The study lasted for 9 weeks.

The baseline rats received subcutaneous injections of 20mg/kg of Demeclocycline (Sigma Chemical Co., St. Louis, MO) at fourteen days (-14 days), and 10mg/kg of Calcein (Sigma Chemical Co., St. Louis, MO) at four days (-4 days), before sacrifice. All other rats received 90mg/kg Xylenol orange (Sigma Chemical Co., St. Louis, MO) at the beginning of the study, and 20mg/kg of Demeclocycline at fourteen days and 10mg/kg Calcein at four days before sacrifice. The Xylenol Orange label enables us to determine the amount of bone formed during the entire experimental period, and the Demeclocycline and Calcein labels allow us to determine bone formation rates of the last 14 days.

The bone mineral content (BMC) and lean leg mass of the right tibiae (from the proximal end to the distal end of the tibia) were measured by dual-energy X-ray absorptiometry using a bone densitometer adapted for small bone animal research (pDEXA, SABRE, Norland Medical Systems, Inc, Atkinson, WI). The measurements were carried out at baseline and at 4, 8, and 12 weeks in rats anesthetized by an intraperitoneal injection of Ketamine (50mg/kg) and Xylazine (10mg/kg). Throughout the study, the scans were performed with a scan speed of 20mm/s and resolution of 0.5 mm x 0.5 mm.

The quadriceps, gastrocnemius and soleus muscles and the right tibiae and lumbar vertebrae were collected at autopsy. Longitudinal sections of proximal tibiae (PT), cross sections at the tibio-fibular junction of the tibial shafts (TX), and cross sections of the 4th & 5th lumbar vertebral bodies, were all cut to 230μm thickness using a low speed metallurgic saw and then ground to 20μm (PT) and 30μm (TX) for histomorphometric measurement.

Histomorphometry was done using a semi-automatic image analysis system (Osteomeasure, OsteoMetrics, Inc, Atlanta, GA) linked to a microscope equipped for transmitted and fluorescence light studies. Here we report some key indices of bone histomorphometric measurements. They include cortical and trabecular bone mass [total cross sectional area of tibial shaft (T.Ar), trabecular bone area (%B.Ar) of the proximal tibial metaphyses (PTM) and lumbar vertebral bodies (LVB)],

Parameters	Body weights	Muscle weights		
		Quadriceps	Gastrocnemius	Soleus
6 mo. male rats[1]				
ORX+NC vs. Sham+NC	→	→	→	↓5
Sham+RC vs. Sham+NC	↑13	↑12	↑10	↑11
ORX+RC vs. Sham+NC	↑12	↑12	→	↑11
ORX+RC vs. Sham+RC	→	→	↓14	→
ORX+RC vs. ORX+NC	↑13	↑8	↑10	↑8
10 mo. male rats[2]				
ORX+NC vs. Sham+NC	→	→	→	↓5
Sham+RC vs. Sham+NC	↑6	↑8	→	↑7
ORX+RC vs. Sham+NC	↑2	↑8	→	↑5
ORX+RC vs. Sham+RC	↓5	→	→	→
ORX+RC vs. ORX+NC	↑3	↑13	↑5	↑12
10 mo. female retired breeders[3]				
Vehicle+RC vs. Vehicle+NC	↑9	↑6	↑13	↑12
PGE_2+NC vs. Vehicle+NC	↓11	↓19	↓15	↓15
PGE_2+RC vs. Vehicle+NC	→	→	→	→
PGE_2+RC vs. Vehicle+RC	↓4	↓6	↓10	↓11
PGE_2+RC vs. PGE_2+NC	↑6	↑25	↑13	↑16

Note: RC, raised cage; NC, normal height cage; ORX, orchidectomy; [1,2,3], obtain from studies 1-3; ↑/↓, % increased/decreased with raised cage.

Table 1. Body and muscle weight changes.

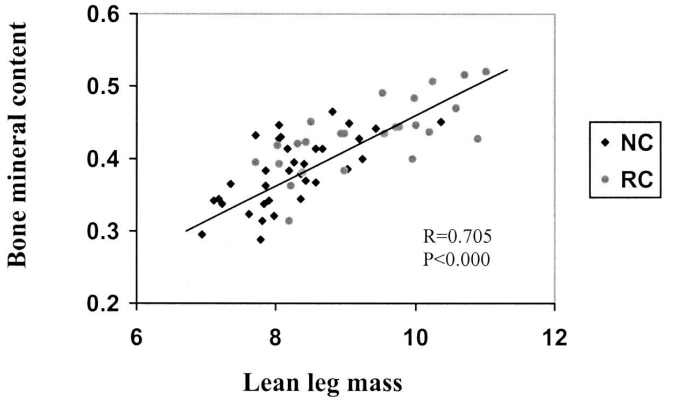

Figure 1. pDEXA changes of lean leg mass and BMC of the right tibiae in normal height cages (NC) and raised cages (RC). Notice that raised cages increased lean leg mass as well as bone mineral content of the right tibiae. Data from 6 and 10-month-old male and 10-month-old female retired breeders.

periosteal bone formation rates of tibial shafts (BF), and bone resorption at the trabecular and endocortical surfaces (BR). The measurements and calculations were carried out according to Parfitt et al[13,14]. We also report the changes in muscle weights.

Results are presented as Means±SD. The statistical analyses were performed using SPSS for Windows (version 10.0, SPSS Inc. Chicago, IN). As not all data were normally distributed, we selected the Kruskal-Wallis test to analyze the differences between groups followed by Mann-Whitney U test for post-hoc comparison. Univariate ANOVA was used to observe the interaction between raised cages and orchidectomy (or PGE_2) using body weights as a co-factor. The two-tail Spearson test was selected to analyze the bivariate correlation between lean leg mass and bone mineral content of the right hindlimbs. $P < 0.05$ was considered significant.

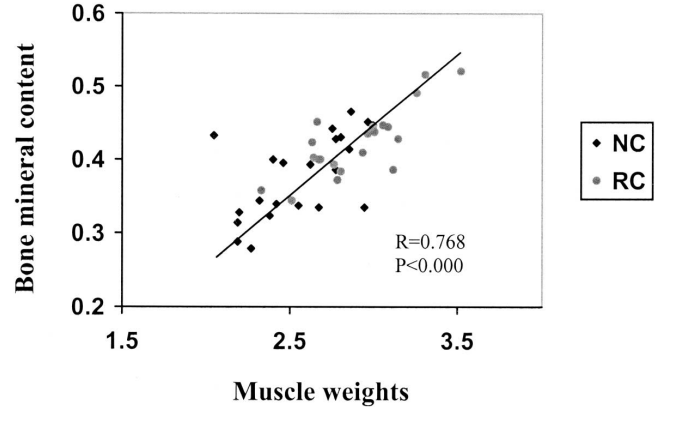

Figure 2. Correlation of BMC as measured by pDEXA and muscle weights (gastrocnemius) of the right tibiae following housing in normal height cages (NC) and raised cages (RC). Notice that raised cages increased muscle weight as well as bone mineral content of the right tibiae. Data from 6 and 10-month-old male and 10-month-old female retired breeders.

Results

Body and hindlimb muscle weights increased with raised cages (Table 1), this accompanied an increase of BMC in the right tibiae (Figs. 1&2). These increases were larger in younger animals than in older ones (Table 1). The increases of body and muscle weights preceded the increase in bone mass, since body and lean leg mass increased an average of 6% at 4 weeks and 12% at 12 weeks, while BMC did not change at 4 weeks, it started to increase at 8 weeks and had increased about 8% at 12 weeks in 6 and 10-month-old male rats (Fig.3).

In intact animals, raised cages increased cortical bone mass (TX) in the 6-month-old male rats (Sham+RC) by stimulating periosteal bone formation (this would represent stimulated bone modeling, Fig.4). Raised cages had no significant effect on cancellous bone mass in the proximal tibia (PTM), but decreased trabecular bone resorption by 35%.

In 10-month-old male (Sham+RC) or female rats, periosteal bone formation of the tibia was increased (Fig.5), but tibial bone mass did not change significantly during the study period (Table 2).

In 6-month-old orchidectomized rats (ORX+RC),

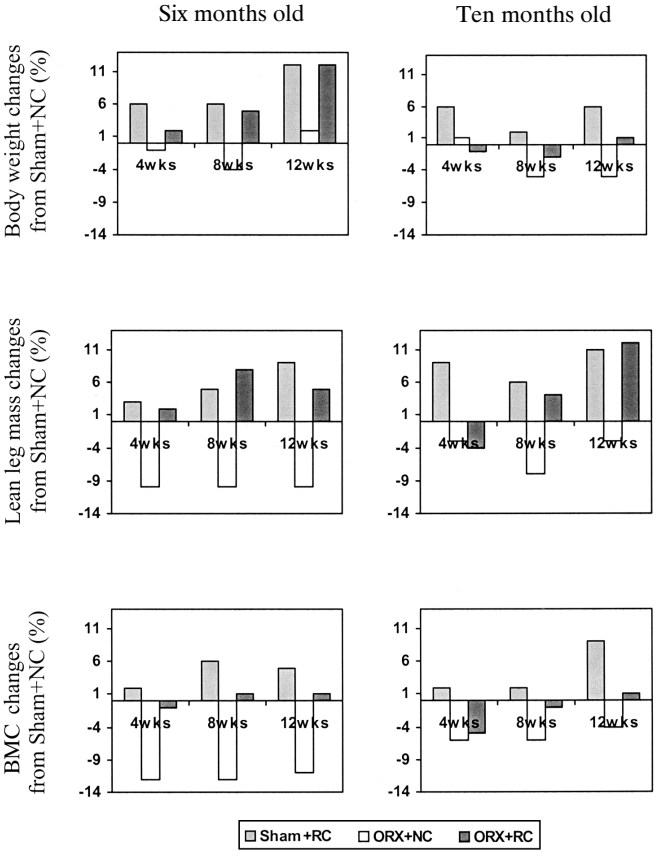

Figure 3. Body weights (A), lean leg mass (B) and bone mineral content (BMC) of the right tibiae (C) data of 6 and 10-month-old sham/orchidectomized male rats. Notice that the increase of body weight and lean leg mass preceded that of BMC, and increased to a large extent that of BMC.

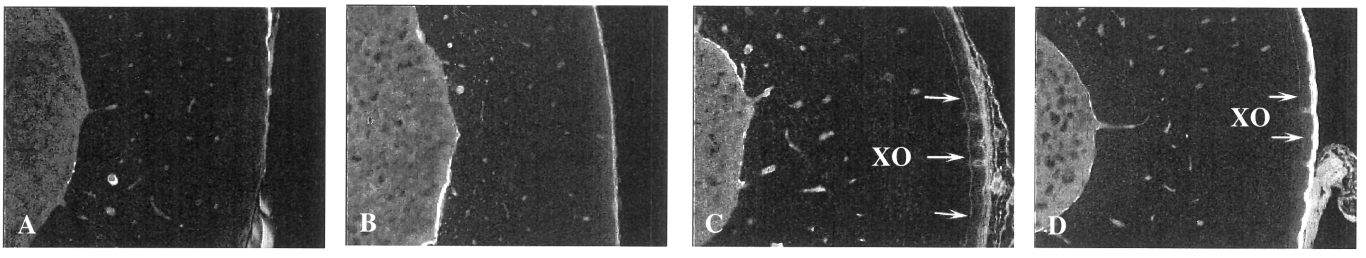

Figure 4. Tibial shafts of Sham+NC (A), ORX+NC (B), Sham+RC (C) and ORX+RC (D) in 6-month-old rats. Notice new bone (bone formed started from Xylenol Orange label (arrows) towards bone surface) was added at the periosteal surfaces in the raised cage animals (B, D), especially in the Sham+RC group (C). Orchidectomy induced thinner cortex (B) and ORX+RC prevented the cortical bone loss (D). XO: Xylenol Orange label. × 50, 20 μm thick Villanueva bone stain sections.

raised cages partially prevented ORX-induced bone loss (ORX+NC) by stimulating periosteal bone (TX) formation and decreasing bone resorption next to the marrow, so raised cages stimulated periosteal bone modeling and depressed bone remodeling at the endosteal surface (Table 2, Fig. 4).

In 10-month-old male orchidectomized rats (ORX+RC), raised cages also tended to reduce endosteal and trabecular bone loss induced by ORX and increased periosteal formation, but these changes were not significant enough to completely prevent ORX-induced trabecular or cortical bone losses.

In 10-month-old female retired breeders, treated with combined raised cages and PGE_2 (RC+PGE_2), both cortical (TX) and trabecular bone mass (PTM) were increased over raised cage (Vehicle+RC) or PGE_2-treated rats alone (PGE_2+NC), that accompanied dramatically increased bone formation at cortical bone periosteal and endosteal surfaces, and trabecular surfaces of cancellous bone, and decreased bone resorption on the endosteal surfaces (Figs. 5-7).

Discussion

In these studies, making rats rise to an erect bipedal stance for feeding improved hind limb muscle mass and increased tibial cortical bone mass by increasing periosteal bone formation. Raised cages also inhibited cancellous bone loss in the proximal tibial metaphysis and prevented net cortical bone loss in tibial shafts in 6-month-old orchidectomized rats. It seems these effects were due to decreasing the bone turnover due to ORX on trabecular and endocortical surfaces (i.e. bone next to marrow) and stimulating modeling-dependent bone formation at the periosteal surface.

However, the osteogenic effects of raised cages in older animals were less pronounced. Therefore, in order to increase bone mass in older animals we used an anabolic agent, PGE_2, which can increase bone mass but is limited in human use because of its side effects when used in larger dosages. To examine the interactions of PGE_2 with mechanical loading by making rats rise to bipedal stance for feeding in raised cages, we used PGE_2 at the level of 1mg/kg/d, which has been reported to be the minimum effective dosage to induce new bone formation and to have minimal side effects[12]. As expected, combined PGE_2 and raised cages (PGE_2+RC) increased cancellous bone mass (PTM) by 10% and cortical bone mass (TX) by 8% accompanied by increases in periosteal, endocortical and cancellous bone formation. These findings suggest the beneficial effects of combining PGE_2 with raised cages. These effects resemble our previous findings that showed that the amount of bone gained during PGE_2 treatment that was greater in heavily loaded sites (i.e. the distal portion of long bone) than in the spine[15]. In combination with various

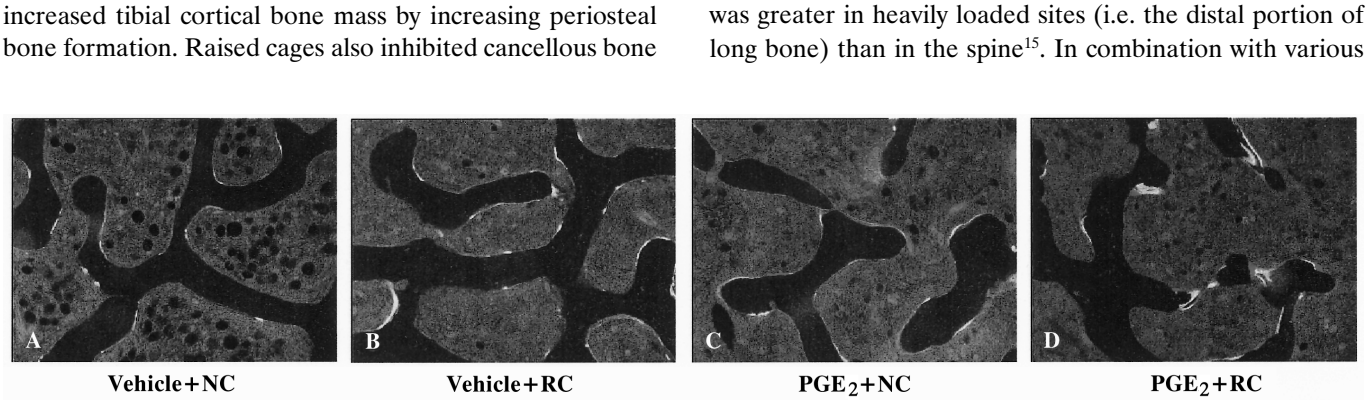

Figure 5. Trabecular bone tissue from lumbar vertebral bodies of Vehicle+NC (A), Vehicle+RC (B), PGE_2+NC (C) and PGE_2+RC (D) animals. Notice more double labeled surface and wider inter-labeled width were seen in the PGE_2+RC group (D). × 50, 20 μm thick Villanueva bone stain sections.

Parameters	Bone mass		Bone dynamics		
	Cortical*	Trabecular#	Cortical-BF (Ps)	Trabecular-BF	Endosteal-BR
6 mo. male rats[1]					
ORX+NC vs. Sham+NC	↓6	↓60	↘34	↗30	↑90
Sham+RC vs. Sham+NC	↑8	↗45	↑20	→	↓35
ORX+RC vs. Sham+NC	→	→	→	→	→
ORX+RC vs. Sham+RC	↘8	↘40	↓24	↘20	↗16
ORX+RC vs. ORX+NC	↑11	↑130	↑60	→	↓50
10 mo. male rats[2]					
ORX+NC vs. Sham+NC	↓5	↘38	↘20	↑90	↑110
Sham+RC vs. Sham+NC	→	↗5	↑20	↗20	→
ORX+RC vs. Sham+NC	↓5	→	→	→	→
ORX+RC vs. Sham+RC	↓7	→	↘20	→	↗20
ORX+RC vs. ORX+NC	↗5	↗35	↑40	→	↓50
10 mo. female retired breeders[3]					
Vehicle+RC vs. Vehicle+NC	→	→	↑14	↑20	→
PGE_2+NC vs. Vehicle+NC	→	→	↑20	↑24	→
PGE_2+RC vs. Vehicle+NC	↑14	↑11	↑50	↑21	↓42
PGE_2+RC vs. Vehicle+RC	↑7	↑23	↑30	→	↓40
PGE_2+RC vs. PGE_2+NC	↑10	↑8	↑40	↑12	↓13

Note: RC, raised cage; NC, normal height cage; BF, bone formation/bone surface; BR, bone resorption; Ps, periosteal; 1-3, data obtained from studies 1-3; *, data of tibial shaft; #, data of proximal tibial metaphyses; ↑/↓, significant % increased/decreased with raised cage; ↗/↘, non-significant % increased/decreased with raised cage.

Table 2. Bone mass and bone dynamic changes.

methods of external loading on the rat tibia shafts, PGE_2 had a synergistic effect on the periosteal bone formation, and had an additive effect on endocortical bone formation[16].

It has been proposed and seems to be true that muscle force is normally the main determinant of postnatal whole-bone strength and bone "mass"[17-20]. In our previous immobilization studies, we found that the loss of muscle weight preceded the bone loss, and recovery of muscle weight preceded the recovery of bone mass[21]. In these studies, we found that lean leg mass and muscle weights (gastrocnemius) of the right tibiae correlated strongly with bone mineral content measured by pDEXA, suggesting that the higher bone mass could relate to the increase of muscle mass. Similar results have been seen in humans[22-25]. Moreover, the wet weight of the quadriceps increased significantly in the raised-cage groups. The quadriceps seems very important in body balance and in preventing falls and fall-related fractures[26], so increasing the quadriceps' strength should be taken into account when considering treatments for bone loss.

When the effects of raised cages are compared to the effects of treadmill running[1-3,27-30], we found they both increased cortical bone mass by increasing periosteal cortical bone formation, and had similar effects on the cancellous

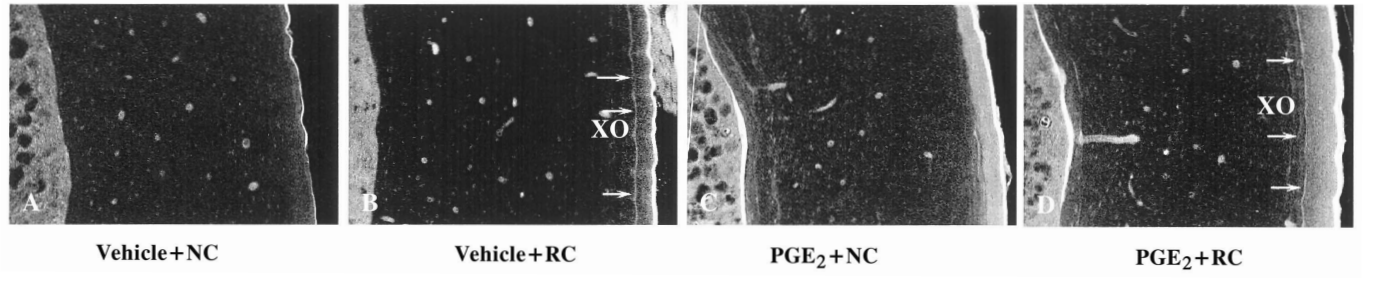

Figure 6. Tibial shafts of Vehicle+NC (A), Vehicle+RC (B), PGE_2+NC (C) and PGE_2+RC (D) in 10-month-old female retired breeders. Notice new bone was added at the periosteal surfaces in the raised cage animals (B, D), especially in the PGE_2+RC group (D). PGE_2 treatment alone (C) also stimulated new periosteal bone formation in the form of poorly stained new bone not taking up pretreatment label of XO. XO: Xylenol Orange label. × 50, 20 μm thick Villanueva bone stain sections.

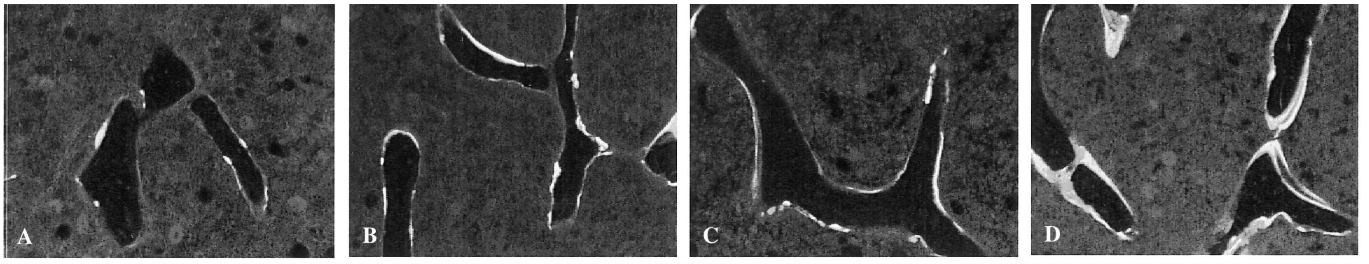

Figure 7. Trabecular bone tissue from proximal tibial metaphyses of Vehicle+NC (A), Vehicle+RC (B), PGE_2+NC (C) and PGE_2+RC (D) animals. Notice more double labeled surface and wider inter-labeled width were seen in the PGE_2+RC group (D). × 50, 20 μm thick Villanueva bone stain sections.

and cortical bone of lumbar vertebral bodies. But raised cages had less osteogenic effects on the cancellous bone of the proximal tibial metaphysis in our male rats than that of treadmill running in female rats (Table 3).

In the future, we will study raised cages' effects on peak bone mass and its interaction with estrogen or estrogen-deficiency and with other anabolic agents (PTH et al). Also, we need to further investigate raised cage effects on vertebrae and the femoral neck, which remain as frequent fracture sites in osteoporosis associated with estrogen deficiency or with advanced aging.

Finally, apart from "bone mass" data, the corresponding bone strength and muscle strength data are necessary to help to better understand the relationships between bone mass, muscle mass, bone strength and other non-biomechanical agents - hormones, anabolic or antiresorption agents, and cytokines et al.

Conclusions

We found that making rats rise to erect bipedal stance for feeding helps to prevent bone loss after orchidectomy, it amplifies the anabolic effects of PGE_2, and it provides an inexpensive, non-invasive and reliable way to increase mechanical loading to certain bones of the rat skeleton.

References

1. Yeh JK, Aloia JF, Chen MM, Tierney JM, Sprintz S. Influence of exercise on cancellous bone of the aged female rat. J Bone Miner Res 1993; 8:1117-1125.
2. Chen MM, Yeh JK, Aloia JF, Tierney JM, Sprintz S. Effect of treadmill exercise on tibial cortical bone in aged female rats: A histomorphometry and dual energy X-ray absorptiometry study. Bone 1994; 15:313-319.
3. Yeh JK, Aloia JF, Chen MM. Growth hormone administration potentates the effect of treadmill exercise on long bone formation but not on the vertebrae in middle-aged rats. Calcif Tissue Int 1994; 54:38-43
4. Umemura Y, Ishiko T, Yamauchi T, Kurono M, Mashiko S. Five jumps per day increase bone mass and breaking force in rats. J Bone Miner Res 1997; 12:1480-1485.
5. Bourrin S, Ghaemmaghami F, Vico L, Chappard D, Gharib C, Alexandre C. Effect of a five-week swimming program on rat bone: A histomorphometric study. Calcif

Attribute	Bone mass		Bone dynamics		
	Cortical*	Trabecular#	Cortical-BF (Ps)	Trabecular-BF	Endosteal-BR
Young rats					
Treadmill Raised cage	↑5[29] ↑8	↑ 23[30] ↗45	↑12[29] ↑20	↑60[30] →	 ↓35
Old rats					
Treadmill Raised cage	↑9[2,3,27] →	↑34[1] ↗5	↑25[2,3,27] ↑20	↑50[1,28] ↗20	↓28[1] →

Note: Young: 1-6 months of age; old: 9-14 months of age; *:tibial shafts; #: data from proximal tibial metaphyses;↑/↓, significantly increase / decrease (%); ↗, non-significantly increase; blank, no data; Ps, periosteal; BF, bone formation/BS; BR, bone resorption; superscript numbers represent the reference number.

Table 3: Skeletal responses to treadmill and raised cage in rats.

Tissue Int 1992; 51:137-142.
6. Grindeland RE, Roy RR, Edgerton RE, Grossman EJ, Mukku VR, Jiang B, Pierotti DJ, Rudolph L. Interactive effects of growth hormone and exercise on muscle mass in suspended rats. Am J Physiol 1994; 267:R316-R322.
7. Newhall KM, Rodnick KJ, Van Der Meulen MC, Carter DR, Marcus R. Effects of voluntary exercise on bone mineral content in rats. J Bone Miner Res 1991; 6: 289-296.
8. Banu MJ, Orhii PB, Wejia W, McCarter RJM, Mosekilde L, Thomsen JS, and Kalu DN. Analysis of the effects of growth hormone, voluntary exercise, and food restriction on diaphyseal bone in female F344 rats. Bone 1999; 25:469-480.
9. Mosekilde L, Thomsen JS, Orhii PB, McCarter RJ, Mejia W, Kalu DN. Additive effect of voluntary exercise and growth hormone treatment on bone strength assessed at four different skeleton sites in an aged rat model. Bone 1999; 24:71-80.
10. Li XJ, Jee WSS. Adaptation of diaphyseal structure with aging and increased mechanical usage in the adult rats: A histomorphometrical and biomechanical study. Anat Rec 1991; 230:332-338.
11. Akamine T, Jee WSS, Ke HZ. PGE_2 prevents disused-induced cancellous bone loss and add extra bone to immobilized rats. Bone 1992; 13:11-22.
12. Jee WSS, Li XJ, and Ke HZ. The skeletal adaptation to mechanical usage in the rats. Cells Mater 1991; 131-142.
13. Parfitt AM, Drezner MK, Glorieux FH, Janis JA, Malluche H, Meunier PJ, Ott SM, and Recker RR. Bone histomorphometry: standardization of nomenclature, symbols and units. Report of the ASBMR Histomorphometry Committee. J Bone Miner Res 1987; 2:595-610.
14. Parfitt AM, Mathews CHE, Villanueva AR, Kleerekoper M, Frame B, and Rao DS. Relationships between surface, area, and thickness of iliac trabecular bone in aging and in osteoporosis. J Clin Invest 1983; 72:1396-1409.
15. Jee WSS and Ma YF. In vivo anabolic actions of prostaglandins in bone. Bone 1997; 21(4):297-304.
16. Tang LY, Raab-Cullen DM, Yee JA, Jee WSS, Kimmel DB. Prostaglandin E_2 increases the skeletal response to mechanical loading. J Bone Miner Res 1997; 12:276-282.
17. Doyle F, Brown J, Lachance C. Relation between bone mass and muscle weight. Lancet 1970; 295:391-393.
18. Burr DB. Muscle strength, bone mass and age-related bone loss. J Bone Miner Res: 1997; 12:1547-1551.
19. Schiessl H, Frost HM, Jee WSS. Perspective: Estrogen and bone-muscle strength and "mass" relationships. Bone 1998; 22:153-156.
20. Frost HM, Ferretti JL, Jee WSS. Perspective: Some roles of mechanical usage, muscle strength, and the mechanical in skeleton physiology, disease, and research. Calcif Tissue Int 1998; 62:1-7.
21. Ma YF, Jee WSS, Yuan ZZ, Wei W, Chen HK, Pun SW, Liang HH, Lin CH. Parathyroid hormone and mechanical usage have a synergistic effect in rat tibial diaphyseal cortical bone. J Bone Miner Res 1999; 14:439-448.
22. Zanchetta JR, Plotkin H, Alvarez-Fihueira ML. Bone mass in children: Noninative values for the 2-20 year old population. Bone 1995; 16:393S-399S.
23. Ferretti JL, Capozza RF, Cointry GR, Garcia SL, Plotkin H, Alvarez-Fihueira ML, Zanchetta JR. Gender-related differences in the relationship between densitometric values of whole-body bone mineral content and lean body mass in humans between 2 and 87 years of age. Bone 1998; 22:683-690.
24. Schiessl H, Willnecker J, Niemeyer GT. Muscle cross sectional area and bone cross sectional area in the human lower leg measured with peripheral computed tomography. In: Third International Congress on Osteoporosis. Xian, China, 1999; pp.79-83.
25. Schönau E. The development of the skeletal system in children and the influence of muscular strength. Hor Res 1998; 12:27-31.
26. Lord SR, Sambrook PN, Gilbert C, Kelly PJ, Nguyen T, Webster IW, Eisman JA. Postural stability, falls and fractures in the elderly: Results from the Dubbo Osteoporosis Epidemiology Study. Med J Austral 1994; 160:684-691.
27. Yeh JK, Liu CC, and Aloia JF. Effects of exercise and immobilization on bone formation and resorption in young rats. Am J Physiol 1993; E182-E189.
28. Swissa-Sivan A, Simkin A, Leichter I, Nyska A, Nyska M, Statter M, Bivas A, Menczel J, and Samueloff S. Effect of swimming on bone growth and development in young rats. Bone Mineral 1989; 7:91-105.
29. Yeh J K, Aloria J F, Chen M M, Koo H C, and Millard W J Effects of growth hormone administration and treadmill exercise on serum and skeletal IGF-1 in rats. Am J Physiol 1994; E129-E135.
30. Iwamoto J, Yeh JK and Aloria JF. Different effect of treadmill exercise on three cancellous bone sites in the young growing rats. Bone 1999; 24:163-169.