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Original Article

Effect of circular motion exercise on bone modeling and bone mass in young rats: An animal model of isometric exercise

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Abstract

The aims of the study are to develop a non-invasive animal model of circular motion exercise and to evaluate the effect of this type of exercise on bone turnover in young rats. The circular motion exercise simulates isometric exercise using an orbital shaker that oscillates at a frequency of 50 Hz and is capable of speeds from 0-400 rpm. A cage is fixed on top of the shaker and the animals are placed inside. When the shaker is turned on, the oscillatory movement should encourage the animals to hold on to the cage and use various muscle forces to stabilize themselves. Rats at 8 weeks of age were trained on the shaker for 6 weeks and static and dynamic histomorphometric analyses were performed for the proximal tibial metaphysis and the tibial shaft. The exercise resulted in no significant effect on animal body weight, gastrocnemius muscle weight and femoral weight. Although the bone formation rate of cancellous and cortical periosteum was increased by the exercise, trabecular bone volume was decreased. The exercise increased periosteal and marrow perimeters and the cross-sectional diameter of cortical bone from medial to lateral without a significant increase in the cortical bone area. These results suggest that circular motion exercise under force without movement or additional weight loading will cause bone-modeling drift with an increase in bone turnover to reconstruct bone shape in adaptation to the demand in strength. Since there is no additional weight loading during circular motion exercise, the net mass of bone is not increased. The bone mass lost in trabecular bone could possibly be due to a re-distribution of mineral to the cortical bone.

Keywords: Mechanical Loading, Cancellous Bone, Cortical Bone, Bone Formation, Bone Resorption

Introduction

The best protection against age-related bone loss and subsequent increased risk of fracture is considered to be the achievement of maximal bone mass at skeletal maturity[1,2]. It has been hypothesized that the influence of exercise (loading) on bone modeling and remodeling is the primary means by which the structural competence of bone architecture is established and maintained[3-5]. Physical activity has long been linked with an increase in bone mass and recently it has been illustrated to have a definitive positive effect on the growing skeleton[3,6-8]. In addition to its positive effects on the immature skeleton, moderate endurance exercise has been shown to increase signs of bone collagen turnover and alter calcium homeostasis in young adult and elderly women[9,10]. Similarly, several animal studies involving young and mature or aging rats have demonstrated that increased physical activity leads to an increase in bone mass[11-14].

Muscle strengthening exercise in general has been classified into three categories: isotonic, isokinetic and isometric[15]. Both isotonic and isokinetic exercise involve movement whereas isometric exercise is static and involves the application of force with no movement. During isometric exercise muscle tension is increased by the application of forces against stable resistance. Unlike with isotonic and isokinetic exercise, there is no movement of the joint and the length of the muscle remains unchanged with isometric exercise. However the application of force without movement does increase muscle strength[16,17].

Many animal models have been developed to simulate human exercise such as treadmill running[11,14,18], swimming[19], weight bearing[20] and jumping[21], all of which are generally not considered isometric exercise. These forms of exercise except swimming, differ from isometric exercise in the fact that they

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not only involve movement but they also involve vertically applied gravitational forces on the skeleton. This fact and the fact that no non-invasive animal model to simulate isometric exercise has been described heretofore emphasize the importance of evaluating this form of exercise and the impact that it has on bone. Here we describe an animal model that simulates human isometric exercise without invasive procedures. It involves placing the animals in a cage attached to the top of an orbital shaker. When the machine is turned on it oscillates in a circular motion and encourages the animals to hold on to the cage and use various muscle forces such as abduction and adduction, extension and flexion, etc. to stabilize them. This increases the frequency of muscle contraction patterns that normally help to maintain quadruped balance with minimal or no alteration of muscle length rather than effecting contraction patterns that primarily subserve locomotion. It thereby simulates human isometric exercise in that muscle force is exerted without movement of the limb. The force on the skeleton in this animal model introduced here is applied horizontally in contrast to the gravitational forces that are applied in other human exercise simulations.

The aims of the study are (a), to develop an animal model using a shaking apparatus to encourage a non-invasive circular motion exercise, (b), to evaluate the effect of the circular motion exercise on bone mass and bone turnover in trabecular and cortical bones in rats, (c), to compare the results of circular motion exercise with the results of our previous study of treadmill exercise.

Material and methods

Animal preparation

Eighteen female Sprague-Dawley rats at 8 weeks of age were purchased from Hilltop Animal Care, Inc. (Pittsburgh, PA). They were randomly divided into two groups as sedentary control and exercise groups. The body weight of the rats was monitored weekly and the experimental period was 6 weeks. For circular motion exercise the training was conducted 5 days per week. An orbital shaker that oscillates at a frequency of 50 Hz and is capable of speeds from 0-400 rpm was used as the training device. The stainless steel cage (10 x 16 x 7 inch cage, W x L x H) was mechanically stabilized on top of the shaker. The animals assigned for exercise were allowed at least a two day acclimation period. During the first 2 days, the speed of the shaker and duration of each running session were gradually increased from 70 rpm for 5 minutes to 150 rpm for 45 minutes. During the first two days adaptation period of training, we observed that it required speed above 150 rpm in order to encourage the animals to hold on to the cage, thereafter, the speed and duration were increased gradually. The speed and duration were gradually increased to 350 rpm for 60 minutes each day in the second week, and this speed and duration were maintained for 5 days per week for the rest of the experimental period. This speed and duration were determined based on preliminary trials and are the point at which the animals did not show signs of undue exhaustion. Animals were unable to hold on to the cage when the speed increased above 450 rpm. All rats with and without treatment were given water ad libitum and were allowed free access to a standard pellet chow diet (Rodent Laboratory chow 5001, Ralston Purina). They were housed under local vivarium conditions (temperature 23.8 °C and 12 hours on/off light cycle). Animals were maintained according to the NIH Guide for Care and Use of Laboratory Animals, and animal protocols were approved by the Laboratory Animal Care Committee of Winthrop-University Hospital.

Group	Number of animals	Body Weight (g)	Gastrocnemius Muscle Weight (g)	Femoral Dry Weight (mg)	Femoral Length (mm)
Sedentary	9	265±12.8	1.74±0.14	516±31	33.3±0.6
Exercise	9	266±13.4	1.77±0.16	522±37	33.4±0.6

Values are mean±SD. All differences were tested by Student's t-test.
All the comparison had p>0.05, considered statistically non significant

Table 1. Effects of isometric exercise on body weight, gastrocnemius muscle weight, femoral dry weight and femoral length of the rats

Preparation of specimens

All rats were labeled with 15 mg/kg of demeclocycline intraperitoneally (Sigma Chemical Co., St. Louis, MO) and 8 mg/kg of calcein subcutaneously (Sigma Chemical Co., St. Louis, MO) at 12 days and 2 days, respectively, before sacrifice. They were anesthetized using ketamine 80mg/kg injected intraperitoneally along with 12mg/kg of xylazine, and euthanized by exsanguination. The gastrocnemius muscle of each animal was isolated and weighed. The right femur of each animal was dissected free of soft tissue and dehydrated for dry weight and femoral length. The right tibia was dissected and cut into three equal parts. The right proximal tibia and tibial shaft were fixed in 70% ethanol solution for two days, and immersed in Villanueva Osteochrome Bone Stain (Polysciences, Inc., Warrington, PA) for 5 days. The specimens were dehydrated by sequential changes of ascending concentrations of ethanol (70, 95 and 100%) and xylene and then embedded in methyl methacrylate (Eastman Organic Chemicals, Rochester, NY). Frontal sections of the proximal tibia were cut at 5 um using

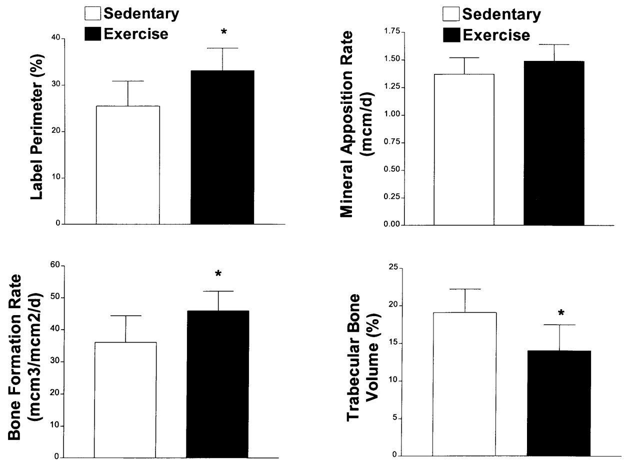

Figure 1. Effect of circular motion exercise on trabecular label perimeter (%), mineral apposition rate (mcm/d), bone formation rate/bone surface (mcm^3/mcm^2/d) and the bone volume (%) of the proximal tibia. Error bars are the S.D. of 9 rats. *Significantly different from the sedentary control group by Student's t-test ($P<0.05$). Notice an increase in the label perimeter and bone formation rate with a decrease in the trabecular bone volume.

a microtome (Leica RM2155, Germany) and cross sections of the tibial shaft proximal to the tibiofibular junction were cut at 40 um using a diamond wire Histo-Saw machine (Delaware Diamond Knives, Inc., DE). All sections were coverslipped with Eukitt (Calibrated Instruments, Inc. Hawthorne, NY) for static and dynamic histomorphometric analysis.

Bone histomorphometry

A digitizing morphometry system was used to measure bone histomorphometry parameters. The system consists of an epifluorescence microscope (Olympus, BH-2), a digitizing pad (Numonics 2206) coupled to an IBM computer, and the morphometry program Osteo Metrics (Osteo Metrics, Atlanta, GA). The interlabel width between calcein and demeclocycline labels beneath the growth plate was used to calculate the longitudinal growth rate. The measured parameters for the cancellous bone included total tissue area, trabecular bone area and perimeter, single - and double - labeled perimeters and inter-label width. These data were used to calculate the percent cancellous bone volume, percent labeled perimeter, mineral apposition rate, and bone formation rate/bone surface according to the standard nomenclature described by Parfitt et al.[22]

In the present study, the regions of cancellous bones measured were 1-4 mm distal to the growth plate for the proximal tibial metaphysis. The measured parameters for the periosteum of the tibial shaft were periosteal perimeter, single- and double-labeled perimeters and inter-label width. These data were used to calculate the percent labeled perimeter, mineral apposition rate, and bone formation rate/bone surface.

Statistical analysis

All data are presented as the means and standard deviation (S.D.) in tables and figures. The Student's t-test was used to examine the difference between the two groups. A significance level of $P<0.05$ was used for all comparisons.

Results

Body weight, gastrocnemius muscle weight and femoral dry weight and length did not differ between the two groups (Table 1). The body weight gain and longitudinal growth rate also did not differ between the two groups (data not shown). Figure 1 shows that circular motion exercise resulted in a significantly higher trabecular bone formation rate/bone surface, but a lower trabecular bone volume than that of the sedentary group ($P<0.05$). The increase in trabecular bone formation rate/bone surface was due to increased label perimeter without a significant effect on the mineral apposition rate.

Figures 2 and 3 show that the exercise training also resulted in a significantly higher bone formation rate/bone surface in the periosteal and endocortical surfaces of cortical bone as compared to the sedentary group. In contrast to the trabecular bone, this increase in both periosteal and endocortical surfaces was primarily due to an increase in the mineral apposition rate, but not the label perimeter. Eroded surface, used as an index of bone resorption in the endocortical area, was also higher in the exercised group in comparison to the sedentary group. Thus, the exercise training increased the bone turnover in the endocortical area.

Figure 4 shows that the total area and marrow area of tibial shaft were both higher in the exercised than in the sedentary group. The cortical bone area did not differ between the two groups, but the shape of the tibial shaft was changed. The width of tibial shaft from medial to lateral was higher in the exercised than that of the sedentary group. The width from anterior to posterior did not differ between the groups.

Discussion

To our knowledge there is no suitable non-invasive animal model that simulates human isometric exercise. The present animal model introduced uses circular motion to encourage the animals to hold on to the cage. Although the "floor" of the cage was moving, the animals used various muscle forces to stabilize themselves. Since there was no movement of the limbs but there was muscle force exerted (application of force with no movement), this model could be considered a close simulation of isometric exercise. The animals do periodically move to adjust their position, however the majority of their exercise time is spent in a stationary holding position. Since it is a circular motion, the forces in this model are generally applied horizontally which allows us to examine the bone adaptation to the direction of forces other than that from gravitational force.

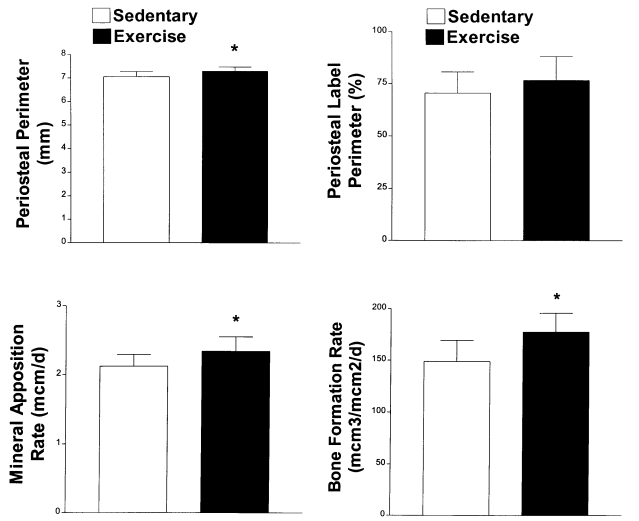

Figure 2. Effect of circular motion exercise on periosteal perimeter (mm), label perimeter (%), mineral apposition rate (mcm/d), and the bone formation rate/bone surface (mcm^3/mcm^2/d) of the tibial shaft. Error bars are the S.D. of 9 rats. *Significantly different from the sedentary control group by Student's t-test (P<0.05).

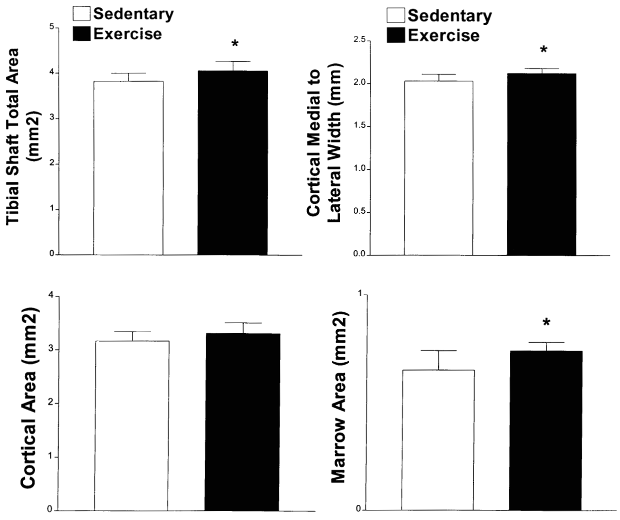

Figure 4. Effect of circular motion exercise on tibial shaft total area (mm^2), Cortical medial to lateral width (mm), Cortical area (mm^2), and the marrow area (mm^2). Error bars are the S.D. of 9 rats. *Significantly different from the sedentary control group by Student's t-test (P<0.05). Notice a change in bone shape and size without a significant increase in the cortical bone volume.

The animals used in this study were young growing rats. Gain in bone size and mass were expected to be rapid in both exercise and sedentary groups. The growth rate, reflected in weight gain over the experimental time period, did not differ between the groups. Since the aim of the study was to compare the difference in bone mass and bone formation between the two groups instead of the rate of growth, the basal control group was omitted. In young growing rats, longitudinal growth is generally sensitive to gravitational mechanical loading such as treadmill training. Moderate exercise with treadmill training may stimulate longitudinal bone growth[23], whereas high-intensity training may delay or stop long bone growth[24]. Other kinds of exercise related to gravitational loading, such as jump training[21] and voluntary running exercise[25] using rats as an animal model also stimulate long bone growth. In this exercise study with horizontal forces, bone length and longitudinal growth rate measured using demeclocycline and calcein labeling were not significantly different between the exercise and sedentary groups, indicating that this type of horizontal force does not enhance longitudinal bone growth.

The circular motion exercise training does however activate the bone formation rate in both the cortical bone and trabeculae of the tibia. Since the area of trabecular bone of the proximal tibia measured was in the metaphyseal region, an increase in the bone formation rate accompanied by a net loss of bone volume in comparison with the sedentary group suggests that the bone resorption rate was activated and exceeded the formation rate. However, findings in the endocortical bone turnover parameters shows that both the eroded surface and bone formation rates were increased and the net marrow area was enlarged in the exercised group as compared to the non-exercised group. The modeling gain in the periosteum induced by the exercise compensated for the net loss in endosteum and results in no significant increase in cortical bone mass.

The most popular method of inducing bone hypertrophy and increasing bone density is progressive overload exercise such as weight-bearing exercise[26,27]. Examining rat tibia (major weight-bearing site) vs the lumbar-5 vertebra (minor weight-bearing site) in response to treadmill exercise, it has

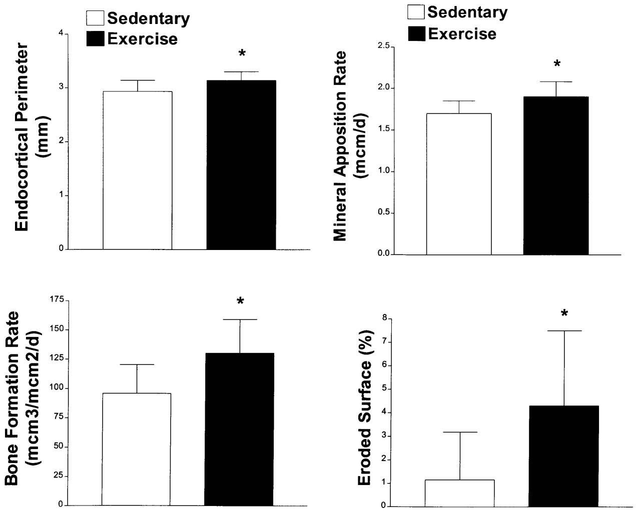

Figure 3. Effect of circular motion exercise on endocortical perimeter (mm), mineral apposition rate (mcm/d), bone formation rate/bone surface (mcm^3/mcm^2/d) and the eroded surface (%) of the tibial shaft. Error bars are the S.D. of 9 rats. *Significantly different from the sedentary control group by Student's t-test (P<0.05). Notice an increase in both bone formation rate and the eroded surface.

	Isometric Exercise	Treadmill Exercise
Body weight	NS	↑
Muscle Weight	NS	↑
Trabecular Label Parimeter	↑	NS ↑
Trabecular MAR	NS	↑
Trabecular BFR	↑	↑
Trabecular Bone Volume	↓	↑
Periosteal MAR	↑	↑
Periosteal BFR	↑	↑
Endocortical BFR	↑	NS ↑
Endocortical Bone Resorption	↑	↓
Cortical Bone Volume	NS	↑

Table 2. Comparison of the effects between Isometric exercise and treadmill exercise.

been shown that the beneficial effects were more significant in the long bone than that of the vertebra[14,23,28].

In a long bone, such as the tibia, the cancellous bone increase induced by treadmill exercise is higher in the distal metaphysis than in the proximal metaphysis[23]. Since circular motion exercise increases the frequency of muscle contraction and muscle forces against stable resistance without movement of the joint and the length of muscle, there is no additional weight loading during the exercise. Thus, this study clearly demonstrates that exercise without additional weight loading does not benefit bone gains efficiently.

When the increase in bone formation rate in the trabecular bone of the proximal tibial metaphysis is examined, circular motion exercise induces an increase in the bone formation rate due to increased osteoblastic recruitment (label perimeter) without an increase in the osteoblastic activity (mineral apposition rate). This is in contrast with the results of a treadmill study using rats of the same age[23] where treadmill exercise increases the mineral apposition rate in both trabecular and periosteal cortical bones without a significant increase in the labeled perimeter during the initial training period (Table 2). Treadmill exercise also increases periosteal bone formation without a significant loss of endocortical bone of the tibia. The net balance of trabecular and cortical bone is increased by treadmill exercise. It appears that weight-bearing exercise increases osteoblastic activity and suppresses osteoclast resorption activity and results in a net increase in bone mass[23,27]. Whereas, the non-weight-bearing exercise increases mainly osteoblast and osteoclast cell numbers at Basic Multicellular Units-based remodeling sites and results in a redistribution of the bone (Fig. 2,3,4).

The contrast between the two types of exercise is that treadmill exercise is weight-bearing against gravitational forces and the exercise with circular motion is non-weight-bearing with the force applied horizontally. Since bone has a remarkable adaptability against strains, the shape and size and bone strength are adjusted through bone modeling and remodeling according to the "Minimal Effective Strain range" and the direction of the strain forces[26]. This phenomenon is noticeably revealed in the change in cortical shape induced by the circular motion exercise. When the orbital shaker is set to oscillate in a circular motion the animal uses various muscle forces to stabilize it.

There is a definite interaction between bone and muscle but it remains uncertain how muscle forces exert their effects on bones. The effects of muscle strengthening exercise on bone may depend on a number of factors, such as the frequency with which muscle forces are applied, the rate at which muscle forces are applied, the magnitude of muscle forces, and the accumulated numbers of loading events. It is uncertain which factor is more important than the others, however, the circular motion exercise integrates all of these factors at various degrees. It is interesting to note a report in which muscle force is circumvented and the application of stress on the bone is accomplished through vibration[29]. The authors of this report used a vibration apparatus to investigate the effect of 2g (g = 9.8m/sec) vertical acceleration and a frequency of 50Hertz on the prevention of bone loss in the ovariectomized rat. Although this non-physiological mechanical stimulation is not a muscle strengthening exercise, it exhibits the function of preventing loss of bone mineral density. The mechanism is not clear, however, the authors do suggest that the vibration, with the application of increased stresses on the bone, is responsible for the suppression of ovariectomy induced bone turnover.

Although the mass of gastrocnemius muscle was not affected by the 6 weeks of circular motion exercise, it is certain that loading frequency was increased during the exercise. It is possible that the muscle contraction applies a horizontal force and the bones respond to the stress by increasing cortical diameter, particularly on the medial to lateral side in this case. Our results demonstrate the importance of loading frequency of muscle force in interaction between muscle and bone. In contrast, treadmill exercise involves weight-bearing against gravitational forces and the mechanical loading is distributed evenly along the cortical bone. Therefore there is no significant change in the shape of the tibial shaft.

Conclusion

Using a shaking apparatus, we have developed a non-invasive animal model that simulates human isometric exercise. There is neither additional weight loading nor movement involved during the exercise and the forces are applied horizontally. We found that this exercise enhances bone modeling activity and bone turnover. It also resulted in a reconstruction of the bone shape in adaptation to the demand in strength. Since there is no loading during the circular motion exercise, the bone net mass is not increased as in weight-bearing exercises such as treadmill exercise. These points illustrate the importance of weight loading and the ability of bone to adapt to the orientation of force.

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