Jumping Improve Bone Architecture By About 50% Compared to Running After Hindlimb Unloading in Rats

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ABSTRACT

Exercises are important issue in the treatment of osteopenia and osteoporosis with elimination of several side effects of drugs. Therefore, the aim of the current work is to compare between running and jumping exercise effects on the trabecular bone architecture of both femur and tibiae in adult rats. Furthermore, to quantify the appropriate dose of exercise to treat bone loss and osteopenia developed secondary to hindlimb unloading. Forty Wister rats (20 males and 20 females) divided into four groups (each group n=10): Basal control (BCON), Control (CON), Jumping (JUMP), and Running (RUN). All rats were tail suspended for two weeks to develop osteopenia in their hindlimb femur and tibiae. The BCON group was sacrificed to represent the osteopenic bone architecture. The CON group was left 6 weeks without any intervention while, the JUMP, and RUN groups were treated by exercises for the same period. Histomorphometric assessment including the following parameters: Tissue area (T.Ar): (in µm2), Marrow area (Ma.Ar.) (in µm2), Trabecular area (Tr.Ar) (in µm2), Trabecular width (Tb Wi) (in µm), Cortical width (Ct Wi) (in µm), Trabecular separation (Tb Sp) (in µm), Trabecular bone volume: normalized by tissue volume (BV%TV), Trabecular number (/mm), and Porosity (%). Both Jumping and running improved bone architecture in the left femur by about 25 to 50% respectively, but only jumping exercise improved right but not left tibial architecture by more than 50%. The effect of exercise developed was site specific and type dependent. High impact exercise improved trabecular architecture greater than low impact exercise.

Key Words: Bone Architecture; osteopenia; jumping exercise; treadmill running; adult rats; hindlimb unloading

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INTRODUCTION

Osteopenia defined as less bone than normal when compared with most healthy people of the same age, height, weight, gender, and race [1]. Osteopenia and osteoporosis negatively affecting older population quality of life especially females both physically and socially[2]. Several medications were introduced to treat the bone loss as antiresorptiv e agents and anabolic agents. Antiresorptive agents are widely used for bone loss treatment but had a serious side effect of reducing the overall bone turnover rate[3], gastrointestinal intolerance, and musculoskeletal pain[4]. Additional to medications, regular exercise was used and found improving all aspect of quality of life especially physical aspects. Therefore, exercises are considered an important part in the treatment of osteopenia and

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osteoporosis [5].

Exercises as a therapeutic modality considered a lifelong activity used to prevent, treat and manage several diseases and health problems. Although there were a lot of beneficial effects of exercises on all aspect of human health, the key metabolic changes secondary to exercises and their underlying metabolic changes remain largely unknown. Furthermore, the type, frequency, and intensity of the exercise necessary for acquiring and maintaining optimal health or improving the recovery from a disease also remain unclear [6].

Mechanical stress developed by exercise is considered a determinant of both microscopic and macroscopic structure of the skeletal system of all living creatures. Skeletal unloading secondary to fractures, immobilization and prolonged bed rest in humans has been found to cause profound bone loss and marked architectural deterioration of both cortical and cancellous bones [7].

The jumping exercise was found effective in improving the serum bone resorption in non-athletes young females. The jumping was done for only two weeks with ten jumps per day for five days per week. On the other hand, this jumping regime did not change significantly the bone formation markers indicated by osteocalcin levels [8].

Research ethics and technical difficulties concerning experiments on humans mandate using animal models to test different exercise regimes for several diseases and abnormalities. There were several animal exercise models developed to simulate the physical activities of humans. Animal models permit testing both beneficial and adverse effects of certain exercises to clinical implementation on human subjects. These were proposed due to several limitations of performing human studies including significant cost and difficulty of designing experiments that follow participants for a time period to examine certain outcomes that naturally develop over the lifespan. Rodents have the advantages of a shorter life span than humans, a short gestational period, and many offspring [6].

Hindlimb unloading in rats was developed by the National Aeronautics and Space Administration in USA (NASA) at first to simulate the state of weightlessness state in spaceflights. Since that time at mid-1970, hindlimb suspension was the most commonly used animal model to study bone loss and musculoskeletal disorders as well [9].

Animal models used both aerobic exercise models as treadmill running, wheel running, and swimming and progressive resistance training, or anaerobic exercises as jumping and weight lifting to test their effect on bone microstructure [6]. Bone modeling and remodeling are affected mainly by two major forces; ground reaction forces developed secondary to weight and inertial muscular forces to develop movement [10].

Progressive resistive training in the form of squat jumping with adding loads was used to improve bone recovery after hindlimb unloading in rats. The training program was found effective in improving bone quality regarding total Bone Mineral Content (BMC), total and trabecular volumetric Bone Mineral Density (vBMD), geometry and mechanical strength in the proximal tibia metaphysis. The results indicated beneficial and protective effects of exercises on trabecular strength following subsequent disuse period [11].

The jumping exercise succeeded to retain cancellous bone mass and to improve the trabecular thickness in distal femur of growing rats [12]. Progressive and intensive treadmill running protected hindlimb muscles from atrophy secondary to 4 weeks tail suspension period [13].

A comparison between the treadmill running and jumping exercise was made to test their capability to restore bone loss secondary to tail suspension. The results suggested that both exercises were successful in recovering suspension-induced osteopenia at the growing rat femur [14].

Long-term effects of jumping exercise extended for 32 weeks was studied on the bones of female rats. It was found that eight weeks was the minimum time required to improve tibia bone mass which continued to improve for the remaining 24 weeks. The improvements were greater when the exercises were given three times per week instead of only one time weekly [15].

The effects of exercise for bone loss treatments were largely documented in the scientific literature, but how much it was effective, was not documented. Furthermore, which part of the body is affected more and by which type of exercise still needs a confirmation. Therefore, the aim of the current study was to quantify the effect of treadmill running and jumping on bone loss and osteopenia developed secondary to hindlimb unloading. Furthermore, the second aim is to compare between running and jumping effects on the bone architecture of both femur and tibiae in adult rats.

MATERIALS AND METHODS

The protocol of the current study was approved by the Ethical Committee of Animal care and use of the faculty of medicine (Kasr Al Ainy), Cairo University. All experimental procedures and animal handling were carried out according to the protocol of IACUC (Institutional Animal Care and Use Committee). Forty (20 males and 20 females) Adult (12weeks) Wister rats (weight 121.7± 21gm) were obtained and housed at the biological testing center, Faculty of Medicine, Cairo University at the beginning of the experiment. The rats were housed in well ventilated plastic cages (only one rat per cage) with a stainless steel cover (27 x 42 x 16cm) under controlled environmental conditions of light (12/12 h light/dark cycle). Rats were allowed free access to standard laboratory chow and water all the period of the experiment. The cage bedding was changed three times a week to ensure proper cleaning and avoid infections. The same subject handled all the animals to diminish handling stress during the periods of familiarization and training.

Hindlimb unloading:

Hindlimb unloading technique was used similarly to those previously explained by Holton and Globus, 2001. Briefly, a very small paper clip was used with the appropriate diameter to the tail of the rat. The top tail area was cleaned and sterilized with 70% ethanol and dried well just immediately before tape insertion. About 5cm double face, adhesive tape was placed on the well-cleaned

tail. The paper clip was added to the tape by way of its holder being superior. A galvanized wire was attached to the top of the paper holder and fixed on the metal cover of the cage.

The hind-limbs were extended fully to ensure being unloaded through the total period of suspension (14 days). The head down position, by about 30 degrees prevented the hind limbs of the animals from touching the ground or any walls of the cage to eliminate weight bearing. The animals were able to reach their food and water while being suspended. All animals were checked once daily, for close monitoring of their health, tail circulatory embarrassment skin wound and movement.

Experimental design:

After the end of the suspension, the animals were randomly allocated into four groups as follow:

Group I (n=10): basal control group (BCON) in which the animals were dissected immediately after the end of suspension to represent the osteopenic bone changes secondary to tail suspension.

Group II (n=10): control group (CON) the sedentary group in which the animals were stayed sedentary without exercise intervention, only normal cage activities, until the end of the experiment for six weeks.

Group III (n=10): the Jumping exercise group (Jump) in which the animals were allowed to jump ten times per day for five days/week for a total of six weeks

Group IV (n=10): the Treadmill running exercise group (RUN) in which the animals were allowed to run on a treadmill for 30 min/day for 5days/week for a total of six weeks.

Jumping exercise:

Animals in the jumping group were submitted to the exercise individually. The rat was placed in the jumping container previously prepared. The controller delivered electrical stimulus forcing the rat to jump and reach the top of the tank wall with their forelimbs and climb the container. The rats were returned to the floor of the container to repeat the procedure. After 3-4 days of adaptation to handling and container placement, the jumping was repeated for ten times per session. After 2-3 weeks almost all the rats no longer needed to more than two stimuli to jump all ten times and more than two rats needed no stimulus to jump. At the beginning of the training period the jumping height was very low but, after three weeks of training, the rats were capable of reaching the top of the container. Usually, the exercise takes about 10 min for the total ten rats each day of training.

Treadmill running exercise:

The exercise protocol was performed on a normal standard treadmill at a velocity of 1Km/h (=16.7 m/min) for 30 min per day, 5days/week. The training was performed in the morning (9.00-11.00 am). At the beginning of the experiment, the trained group accomplished about four days of familiarization to treadmill running with a gradual daily increase in time from 5 minutes to 30 min per day. After these four days, the exercise was performed for 30 minutes for the following six weeks.

Animal scarification:

At the end of the experiment, rats were killed by a lethal dose of inhaled ether. The left femur and left and right tibiae were excised and kept in 70% ethanol and prepared for histological analysis.

Tissue preparation for histological sections:

Immediately after harvesting, the left femur, the right, and left tibiae were cleaned carefully from adherent soft tissues and muscles and immersed in 70 % ethanol and transferred to the laboratory to be processed for histological analysis. The proximal tibia and distal femur were sawed and sectioned for micro technique preparation. The histological processing included;

1. Fixation (70 % ethanol) at room temperature
2. Decalcification by formic acid for about 15 days (softening process)
3. Dehydration in graded ethanol solutions
4. Clearing in xylene
5. Embedding in paraffin blocks
6. Sectioning, 10µm sections were cut.

The sections were taken longitudinally by microtome in the frontal plane for tibia specimen and sagittal plane for femur specimen. The blocks were divided into 2 zones; medial and lateral for femur specimen and anterior and posterior for the tibia specimen. The sections were obtained by a mean of 4 sections (4-5 range) in each zone. The total number of slides was three slides with 8-9 sections mounted over it for each bone sample. The total number of sections was about 1200 for the overall three bones in all experimental groups with a total of about 400 sections for each bone.

Slides were dewaxed in xylene, rehydrated with graded alcohol solutions, cleared in phosphate-buffered saline (PBS) and then stained with Hematoxylin and Eosin (H&E). Stained slides were then dehydrated in alcohol, cleared in xylene, and mounted with DPX (Distyrene, Plasticizer, and Xylene mixture) and coverslips.

Histomorphometric analysis:

Histomorphometry was performed according to the recommendations, symbols, and nomenclature of the American Society for Bone and Mineral Research [16]. Histomorphometry was performed on four sections/ bone, and a section with artifacts was excluded from the analysis. The total number of section analyzed was 533. The researcher was blinded to the name or type of group for which the rat belonged.

The histomorphometric parameters assessed included:

Tissue area (T.Ar): (in μm^2), Marrow area (Ma.Ar.) (in μm^2), Trabecular area (Tr.Ar) (in μm^2), Trabecular width (Tb.Wi) (in µm), Cortical width (Ct.Wi) (µm), Trabecular separation (Tb.Sp) (in µm), Trabecular bone volume: normalized by tissue volume (BV%TV), Trabecular number (/mm), and Porosity (Po) (in %).

Statistical analysis:

All data are expressed as means ± SD. The statistical analyses were performed using the IBM SPSS Statistics 23 software package for Windows. Comparisons between groups were performed by one-way analysis of variance followed by Bonferroni's posthoc analysis. The statistical significance level was set at $p < 0.05$.

RESULTS

No rat died during the overall course of the experiment. Weight gain was similar within each experimental group and between the four groups ($p<0.05$).

Bone histomorphometric analysis:

Hindlimb unloading by tail suspension developed profound structural deterioration of the distal femur and proximal tibia trabecular bone. Both jumping and running exercises improved bone architecture but in a different way and specific sites.

Left femur microarchitecture:

Running decreased porosity related parameters as Marrow area (Ma Ar), trabecular separation (Tb Sp), and porosity index (Po) in the distal femur not in the proximal tibiae. Marrow Area decreased in the RUN group compared to BCON ($p<0.025$) and CON ($p<0.004$) by about 21% and 27% respectively. Trabecular separation decreased in RUN group compared to CON ($p<0.015$) by about 33%. The porosity decreases significantly in the RUN group related to BCON group ($p<0.025$) and CON group ($p<0.004$) by about 21% and 26% respectively. On the other hand, both running and jumping exercise improved bone related parameters of the distal femur including trabecular width (Tb Wi), trabecular bone volume (BV%TV), and trabecular bone area (Tr Ar). Trabecular bone volume and area increased significantly in RUN group compared to BCON ($p<0.025$) and CON group ($p<0.004$) by about 29% and 44% respectively. Trabecular width increased significantly in the JUM group when compared to BCON group ($p<0.000$) and CON group ($p<0.019$) by about 48% and 18.5 % respectively (fig.1).

Right tibia microarchitecture:

Contrary to the left femur, jumping exercise improved bone related parameters and decreased porosity related parameters in the proximal right tibia. Marrow areas decreased in the JUMP group when compared to BCON group ($p<0.019$) and CON group ($p<0.01$) by about 33% and 31% respectively. Porosity decreased significantly in the JUMP group when compared to BCON group ($p<0.019$) and CON group ($p<0.010$) by about 33% and 31% respectively. Trabecular bone volume (BV%TV) and trabecular area (Tr Ar) increased significantly in the JUMP group when compared to BCON group ($p<0.017$) and CON group ($p<0.002$) by about 45% and 52% respectively (fig.1).

Left tibia microarchitecture:

Left tibia unlike right one improved bone related parameter and decreased porosity related ones but failed to reach significant levels. The trabecular number and cortical width did not affect significantly by either running or jumping exercises.

DISCUSSION

The main result of the present study is that both high impact jumping exercise and endurance running exercise succeeded to retain bone microstructure after suspension-induced osteopenia in adult rats. The response of bone to the exercise was selective in the way that femur affected more by the running exercise while on the other hand right tibia affected more by jumping exercise but left tibia is not affected at all.

The selectively in the response of bones to the exercise referred to the way the exercise load the skeletal system. The normal anatomical acute flexion of the rat knee suggested dissipating a greater amount of ground reaction force developed at the jumping impact which decreases the amount of force transmitted to the femoral bone. The force dissipation may explain way tibia is affected significantly than femur by the jumping exercise. However, the next question is why right tibia affected rather than the left tibia. The answer may be explained partly by a recent work suggested that eight weeks of jumping exercise is the minimum time required to improve the tibia bone mass [15]. The current work extended for only six weeks which is the sufficient time to develop significant improvement of right tibia bone architecture but not the left.

Running exercise improved significantly bone architecture in the femur rather than tibia which may be explained by the nature of walking pattern of the rat. The velocity of running used in the current work is considered moderate velocity through which the animal must increase its speed higher than its normal walking pattern. The higher velocity force the rat to either increase its stride length, therefore, increase the stance time or increase the stride frequency. Both mechanisms were observed alternatively but not necessarily in an equal manner. A previous work suggested that the range of speed used in the current study (about 40cm/second) increased the frequency of the strides linearly [17]. Muscles act during normal locomotion in only three normal modes; shortening, the same length, and stretching. Muscle work to stabilize the limb during certain locomotive activity which intern store energy inside it to be liberated in the succeeding phase of shortening. The energy stored inside the muscle is explained as a spring type mechanism and it is developed basically by the animal to minimize the energy expenditure by the muscles [18].

The spring mechanism developed during higher speed of running suggested in the current study to be developed by Calf muscle over the distal femur. This spring developed greater tensile force over the muscle attachment site at the distal femur could explain the improvement observed in the running group at the bone architecture of this area. Furthermore, the increase in the speed of walking forces the rat to move faster by increasing the swing phase and rapidly translating its legs to start another stride. The rapid translation suggested induced stress over the distal femur by shortening of the Calf muscle to clear the foot off the ground. This alternative stretch-shorten pattern of moving musculatures allow the muscle-tendon unit of the animal to optimize its performance through optimal utilization of stride frequency and length [19]. Confirmation of the suggestion may be recommended for future work to analysis the muscle activity during the gait cycle.

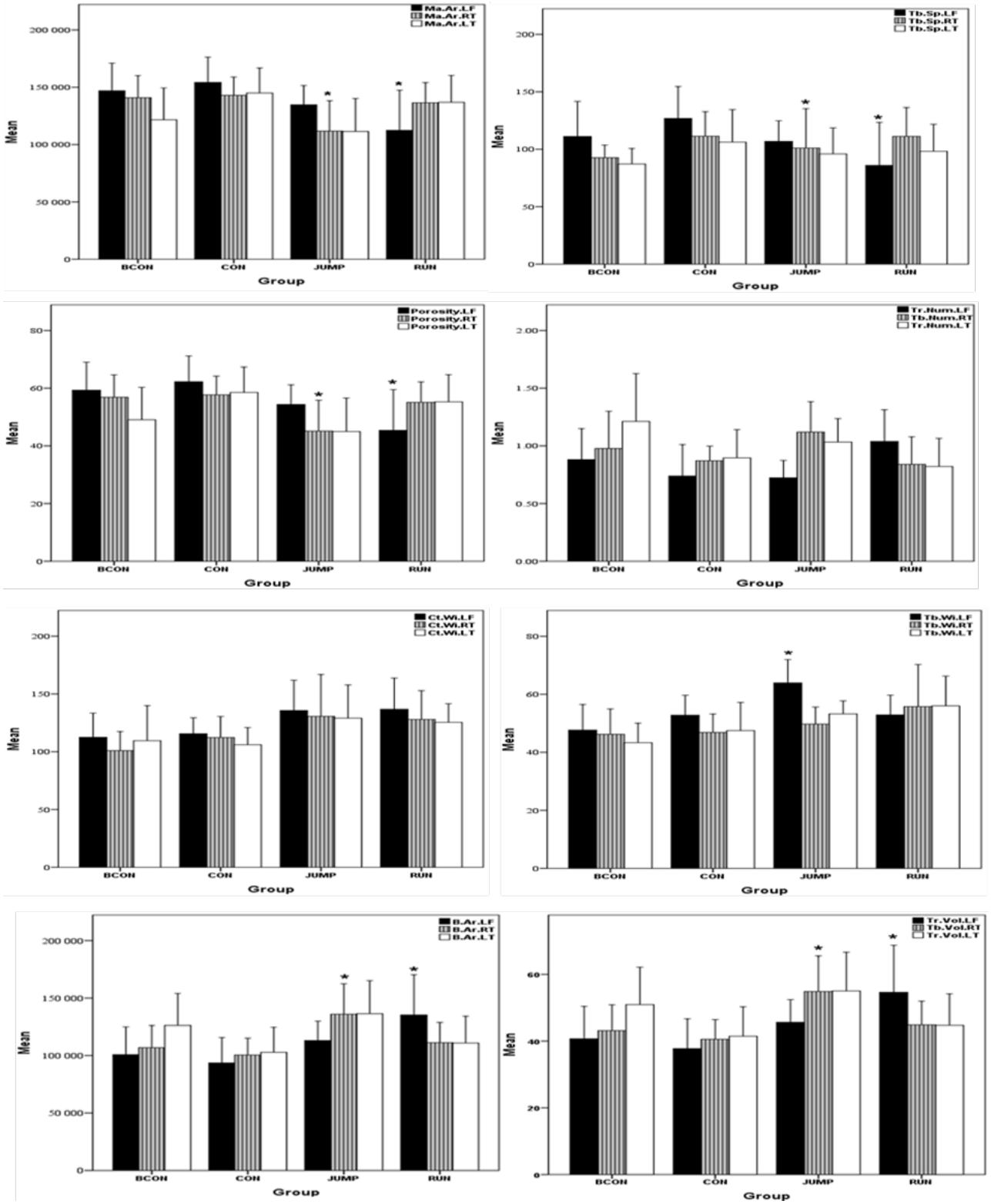

Fig1. Bone histomorphometric parameter comparison between the four groups; BCON, CON, JUMP and RUN. The values expressed as the mean± standard deviation, $p<0.05$ for the total three bones tested; LF (left femur), RT (right tibia), and LT (left tibia)

Similar results obtained by several studies on the positive effect of jumping during, but not after the period of tails suspension. The jumping frequency was 30 jumps/day, 5days/week for only three weeks. The exercise succeeded to retain cancellous bone mass and improving trabecular thickness without significant improvement in the trabecular number [12]. The trabecular number also did not improve in the current study in all exercise groups either jumping or running. The results may be attributed to the notion than the number of primary trabeculae developed during growing may be thickened secondary to exercise and thinned secondary to disuse but lost one

couldn't be replaced [20]. Current work used adult rats, therefore, the primary trabeculae numbers kept unchanged.

It was found that progressive and intensive treadmill running protected hindlimb muscle from atrophy secondary to 4 weeks suspension period experiment. The maximum protection against muscle atrophy was observed in histochemical type I (slow twitch, oxidative metabolic) muscle (soleus vastus intermedius, and adductor longus). The great improvement of soleus muscle mass in the suspended and exercised rats was further investigated for myofibril protein content which was found to be significantly lower than that of the control group [13], [21].

Similar results were obtained by a recent study compared between jumping and running exercises developed similar improvements in the trabecular width of the distal femur of the growing rats. Histomorphometric findings measured by MicroCT has been found increased Tb.Th in jumping exercise group while Tb.N has been improved in treadmill exercise group, Jumping improved the trabecular architecture by increasing the thickness of the existing trabeculae while on the other hand treadmill running improved the architecture by adding new trabeculae and improving their connectivity [22]. The results explained the normal facilitated response of the growing bones to exercises by adding new bones unlike the current study on the adult rats.

These results suggested that functional adaptation of the trabecular architecture to the loading environment is dependent on several factors as strain rate and magnitude, cycle number and loading direction. The amount of time required for the bone to be totally recovered after the tail suspension was still not clear. The femoral BMD was found recovered after five weeks of training while on the other hand, the trabecular architecture has been found not completely recovered compared with age-matched rats [22].

The degree of recorded improvements in bone architecture secondary to treadmill running exercise ranged from 20-33% for porosity related parameters and 30-44% for bone related parameters. Jumping exercise improved bone related parameter by about 20-48% than both control groups (BCON, and CON). The right tibia improvement secondary to jumping exercise reached about 30-33% for porosity related parameters and about 45%-52% for bone related parameters.

The positive effect of exercise on bone mass and strength has prompted many clinicians and health related professional to recommend to the individual to daily exercise. The purpose of exercise recommendation was to decrease the incidence of osteoporotic fractures and related health problems. The great and still unanswered question was which type of exercise considered more effective and how often should we exercise to positively affecting bone health. The current work may be considered a part of the answer of the previous question.

The result of the current study further confirms the site-specific effect of exercises. Therefore designing an exercise program to strengthen certain bone area must be related directly to the muscle and movement in the desired skeletal site.

If the rat studies on bone strength were confirmed in the human populations, a BMD may not be the best indicator for measuring exercise effectiveness. Therefore, further indicators including bone shape, size and architecture must be suggested to test the effect of exercises on the bone strength. Recommended for future work to extend the periods of exercise to confirm the previous results. The percentage of change introduced by the current work needs further confirmation and support by testing the exercise effects on a large sample size to establish an estimate of the efficacy of exercises on different skeletal areas.

CONCLUSION

The jumping exercise was more effective to restore bone loss secondary to rats hindlimb unloading in left femur and right tibia but not at the left tibia. Running exercise improved left femur micro-architecture but not tibiae. The degree of improvement of bone micro-architecture ranged from about 20 to 40% for the running exercise and about 30 to 50% for the jumping exercise. The bone loss area must be detected accurately and a specific exercise regime should be designed to load the specific area to ensure successful results.

List of abbreviations:

BMD: Bone Mineral Density
BMC: Bone Mineral content
vBMD: volumetric Bone Mineral Density
BCON: Basal Control group
CON: Control group
RUN: treadmill Running exercise group
JUMP: Jumping group
PBS: Phosphate-Buffered Saline
H&E: Hematoxylin and Eosin stain
DPX: Distyrene, Plasticizer, and Xylene mixture
T.Ar: Tissue Area in μm^2
Tr.Ar: Trabecular Area in μm^2
Ma.Ar: Marrow Area in μm^2
Tb.Wi: Trabecular Width in µm
Ct.Wi: Cortical Width in µm
Tb.Sp: Trabecular Separation in µm
BV%TV: Trabecular Bone Volume in %
Po: Porosity in %
MicroCT: Micro Computed Tomography

AUTHOR'S CONTRIBUTION:

MMI carried out experimental work, analysis, data acquisition, interpretation of data and drafted the manuscript. BGE carried out supervision and critical revision of the manuscript. HAI supervised on histological preparation and analysis. All authors read and approved the final version of the manuscript.

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