Original Article

Aerobic Exercise: A Potent Method to Improve Morphology of Bone

Aerobic Exercise and Morphology of Bone

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ABSTRACT

Objective: To study the effect of aerobic exercise on histomorphometery of bone.

Study Design: Experimental study

Place and Duration of Study: This study was conducted in Ziauddin University, Karachi from February 2021 to April 2021 for a period of 8 weeks.

Materials and Methods: The study samples were 12 male Sprague Dawly Rats (SD) weighing 200-300gms, divided into two groups; 6 assigned to the experimental group and 6 to the control group. Data was collected through Maze Engineers Multilane rat treadmill and Nikon INTENSELIGHT C-HGFI. Data analyzation was done using SPSS version 25. Skewness and Kurtosis tests were applied in order to check the normality of data. Independent T-test was applied between both the groups to determine mean values on the similar outcome measures and a p-value of <0.05 was considered to be significant**.**

Results: The study observed a statistically significant increase in Haversian canal diameter, increase in area in the post-exercise group of rats, an increase in the number of lamellae per osteon with exercise and a greater average osteon area in the exercise group. However, a statistically significant difference could not be observed in the number of osteon per high power field (HPF) between the two groups.

Conclusion: Findings of this study suggest that weight-bearing exercises such as treadmill running help increasing the density and strength of the cortical bones, amplifying their morphology. Thus, the study concluded that seven weeks of aerobic training results in improved bone morphology parameters.

Key Words: Aerobic Exercise, Bone Health, Bone Morphology

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INTRODUCTION

Bone health is a major concern of the modern era. Due to increasingly sedentary lifestyles being adopted, degenerative changes in bone are becoming a more common feature of modern times.[1] Aerobic exercises in particular are considered to have a positive influence deteriorating bone health. Bone health starts declining when there is greater bone absorption as compared to bone formation resulting in diminished cortical width reduced trabecular volume.[2]

Animal studies have demonstrated that aerobic exercises such as treadmill running and swimming help to preserve the architecture and composition of bone eventually resulting in overall increased bone strength; a process called “remodeling”. Studies on young mice and rats have shown to increase bone mineral density and periosteal thickness. [3, 4]

During remodeling, key cells known as osteoclasts, osteoblasts and osteocytes perform a role in maintaining the homeostasis of the bone. Osteocytes are the most abundant type of cells which sense mechanical loads placed on the bone.[5] Osteoblasts are bone forming cells responsible for production of collagen type I and they mature to become osteocytes which regulate the composition of bone during mechanical strains. Osteoclasts on the other hand are responsible for reabsorption of damaged bone components.[6]

Bone is composed of organic and inorganic components. The inorganic components of bone consist of hydroxypeptides, calcium, fluoride and sodium ions. The organic components of bone mainly consist of collagen type I and some percentage of non-collagenous proteins.[7] The quantity of collagen has a direct influence on the mechanical integrity of bone. Type I collagen is found to be the most important structural component of the extracellular matrix responsible for the mineralization of the bone.[8] Alteration in crosslinking of these collagen fibers in the extracellular matrix of bone occurs due to lack of physical activity, old age, osteoporosis and osteopenia which may eventually result in bone deterioration

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leading to loss of bone mass.[9] Decreased mechanical competence due to low bone strength in response to decreased minerals such as collagen and number of lamellae may result in increased risk of fractures.[10]

Bone mass can be increased by mechanical stimulation. This includes increased loading on the bone resulting in increase in the bone matrix and other structural components of bone.[11]

Aerobic exercise such as treadmill running can induce mechanical loading stress on the bone. This loading regulates osteoblastic differentiation by bone matrix signaling process. In a rat model study, treadmill training has been shown to increase the cell signaling in bone resulting in increased production of osteoblasts into the bone lamellae showing a direct relationship of exercise in the prevention of bone mass loss.[12] Obesity is one of the factors said to increase bone resorption and decrease bone formation thus resulting in reduced bone mass and strength. Studies conducted on obese rats suggests that involuntary wheel running exercise improves the bone quality by limiting bone resorption.[13] It was histologically noted that treadmill running resulted in increased thickness and number of the bone trabeculae with increase in the cortical bone mass.[14] Hence, this research suggests that increased loading on the bone can improve the bone mass resulting in decreased chances of bone health deterioration.

MATERIALS AND METHODS

This Randomized Controlled Trial was carried out at Ziauddin University, Karachi, Pakistan. The sample was collected by simple random sampling. The subjects were 12 healthy young male Sprague Dawly Rats (SD) weighing 200-300gms, divided into two groups; 6 were assigned to the running/experimental group and 6 to the sedentary/control group.[15]The rats were randomly divided into 2 groups (1 running and other sedentary) experimental and control groups. They were housed in individual cages and fed a standard laboratory stock feed and water. Adequate temperature and day night cycles were maintained. The experimental group was trained for a total of 29 days i-e 7 weeks excluding the weekends. In week 1 they were assigned an exercise program beginning with a 5 minutes warm up of low intensity on a multilane rat treadmill which consisted of a running belt of appropriate size and grip to prevent animal from slippage with a transparent wall in front to enable that the animal watches the surrounding. The warm up time was not included in the exercise session time length. After warm up, the speed was gradually increased to 0.30- 0.34 m/s for 25 minutes/session. Week 2 consisted of 2 sessions per day with speeds increasing up to 0.35-0.45 m/s with a duration of 30-32 minutes/session. Week 3 and 4 consisted of 2 sessions per day with a constant speed of 0.45 m/s for a duration of 30 minutes/session. Following the downhill protocol, 2nd day of week 5 was the day when the rats exercised to the level of exhaustion i.e. speed of 0.55 m/s for 45 minutes/session twice a day. Rest of the days of week 5 continuing into week 6 and 7 the session was conducted once a day. The rate of exercise was 0.30 m/s for 15 minutes.[16] At the end of week 7 after exercising, all rats of both experimental and control group were sacrificed anesthetically, after which bilateral tibia of all the rats were harvested and the transverse section at the level of mid-shaft of tibia was removed from the bone for histological examination. The harvested bone was kept in formaldehyde solution for few hours and then the process of bone demineralization and dehydration using isopropyl alcohol was followed. After the removal of alcohol using xyelene solution, sample fixation and overnight paraffin embedding was done. Finally, 5 micrometer size sectioning of samples was done. The samples were then stained with hematoxylin and eosin dye. 10 slides were made per mouse tibia. Once the slides were prepared we used Nikon INTENSELIGHT C-HGFI microscope with its built in calibrated software to determine the average number of osteons per hpf, number of lamellae per osteon, osteon thickness, Haversian canal diameter and Haversian canal area.

RESULTS

At the end of the 7th week all rats were dissected anesthetically and their bilateral tibia were harvested to study under the microscope. After the preparation of the microscopic slides, both groups were studied under the microscope to assess the post exercise outcomes in exercising group and non-exercising group including the number of osteons/ Hpf, number of lamellae per osteon, area of Haversian canal, diameter of Haversian canal and area of osteon. All these morphological differences can be visually examined in Figure 1.Data was analyzed using SPSS version 25. All variables included in this study were continuous thus these were expressed in mean and standard deviation. Skewness and Kurtosis test were applied to check the normality of the data, values of the test showed that the data was normally distributed, based on this Independent T-test was applied between both the groups to determine mean values on the similar outcome measures. In all calculations, a p-value of <0.05 was considered to be significant. Statistical analysis proved significant difference in mean of the bone parameters of exercising and non-exercising group of rats. When the number of osteon per high power field were compared between the two groups we found that this average count was higher (8.33±1.5) in the exercise group as compared to the control group (6.33±2.25) but the difference was not statistically significant (p-value=0.101). (Table-1). The number of lamellae per osteon showed a significantly greater (p-value=0.002) average number of lamellae in the exercise group (2.79±0.25) than the controls (1.89±0.45). (Table-1). The mean area of Haversian canals of the exercise group also was calculated to be significantly more (p-value=0.001) in the exercise group (99.21±22.56 µm2) as compared to the controls (39.58±12.37 µm2). (Table-1). We found similar results for Haversian canal diameters too. Controls had significantly smaller (p-value=0.001) diameters (6.69±1.42 µm) as compared to the exercisers (10.78±22.56 µm). (Table-1). Lastly, we calculated osteon area found significantly thicker osteons (p-value=0.001) in the exercise group (582.93±106.67 µm2) than the controls (287.37±27.25 µm2) (Table-1).

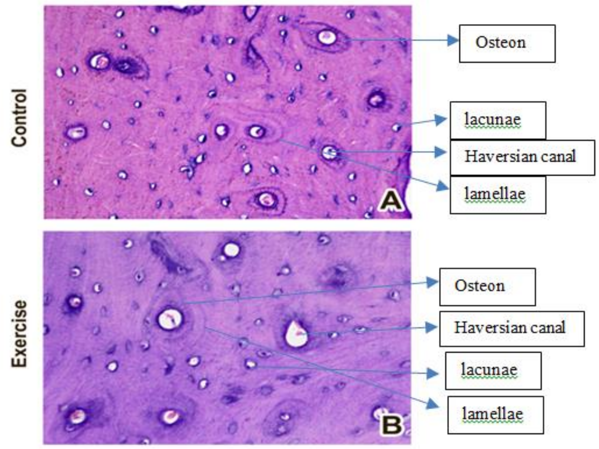

Figure No. 1: Post exercise outcomes in exercising group and non-exercising group including the number of osteons/ Hpf, number of lamellae per osteon, area and diameter of Haversian canal, diameter of Haversian canal and area of osteon.

Table No.1: Post-Training Analysis of the Exercise and Control Group

Variable	Group	N	Mean	Std. Deviation	p-Value
Number of Osteon / HPF	Control	6	6.33	2.25	0.101
	Exercise	6	8.33	1.50	
No of Lamella / Osteon	Control	6	1.81	0.45	0.002
	Exercise	6	2.79	0.25	
Haversian Canal Area (µm^2)	Control	6	39.58	12.37	0.001
	Exercise	6	99.21	22.56	
Haversian Canal Diameter (µm)	Control	6	6.69	1.42	0.001
	Exercise	6	10.78	1.26	
Osteon Area (µm^2)	Control	6	287.37	27.75	0.001
	Exercise	6	582.93	106.67	

DISCUSSION

In this study, we evaluated the histological changes induced by exercise on bone by assessing them visually and quantitatively using histological parameters. We demonstrate that seven weeks of aerobic training results in improved bone morphology parameters. We firstly observed a statistically significant increase in Haversian canal diameter and area in the post-exercise group of rats. This implies greater laying down of bone matrix in response to exercise resulting in increased blood supply to the bone which eventually results in greater diameter of vessels. In response, the matrix on the periphery of the Haversian canals undergoes resorption resulting in increase in their diameters. These findings are supported by those of Hart[17] which highlighted an increase in bone turnover markers. The author further supported our findings by underlining that moderate intensity aerobic training improves bone morphology and aids in slowing or inhibiting osteoporosis by causing a significant decrease in the rate of bone resorption.[17] However, in our study we exhibit direct visualization of these effects on the structural and functional unit of bone. Another important observation was an increase in the number of lamellae per osteon with exercise. It has been implicated by studies carried out previously that tensile strength of bones is directly proportional to the number of lamellae.[18-19] It is also reported that aerobic exercise has a significant impact on the morphology of bone by producing stimulatory signals for osteocytes. As a result, the osteocytes present a response of molecular signaling towards osteoclasts and osteoblasts thus resulting in bone matrix deposition ultimately leading to increased tensile strength. [20] In our study however, we could not find a statistically significant difference in the number of osteon per high power field (HPF) between the two groups. Though the difference being statistically non-significant, we observed that the mean values were greater in the exercise group. This may be due to the fact that minimal strain is sensed by the osteocytes at the front of the tunnel which are involved in laying down new osteon. [21] However, findings of the same study further added that increased mechanical loading in cortical bone leads to more bone remodeling with an increased yet smaller number of osteons and tinier Haversian canals. Finally, we found greater average osteon area in the exercise group. Our findings are consistent with the study of Harding & Beck [22] which revealed that weight-bearing exercises such as treadmill running help in increasing the density and strength of the cortical bones thus amplifying the morphology of bones. However, the study added that as compared to the lower impact aerobics such as walking, higher impact aerobics such as treadmill running yields a more profound effect on the morphology of bones. Moreover, the method used in this analysis was DEXA scan, whereas we directly measured the osteon area histologically.[22]

CONCLUSION

The findings of this study suggest that weight-bearing exercises such as treadmill running help in increasing the density and strength of the cortical bones thus amplifying the morphology of bones.

Author's Contribution:

Concept & Design of Study:	Munira Mukadam
Drafting:	Kevin Joseph Jerome Borges, Sumaira Imran Farooqui
Data Analysis:	Rehan Ahmed Siddiqui, Syed Nudrat Shah, Amna Amir Khan
Revisiting Critically:	Munira Mukadam, Kevin Joseph Jerome Borges
Final Approval of version:	Munira Mukadam

Conflict of Interest: The study has no conflict of interest to declare by any author.

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