Research article

Swimming enhances bone mass acquisition in growing female rats

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Abstract

Growing bones are most responsive to mechanical loading. We investigated bone mass acquisition patterns following a swimming or running exercise intervention of equal duration, in growing rats. We compared changes in bone mineral properties in female Sprague Dawley rats that were divided into three groups: sedentary controls (n = 10), runners (n = 8) and swimmers (n = 11). Runners and swimmers underwent a six week intervention, exercising five days per week, 30min per day. Running rats ran on an inclined treadmill at 0.33 m.s⁻¹, while swimming rats swam in 25°C water. Dual energy X-ray absorptiometry scans measuring bone mineral content (BMC), bone mineral density (BMD) and bone area at the femur, lumbar spine and whole body were recorded for all rats before and after the six week intervention. Bone and serum calcium and plasma parathyroid hormone (PTH) concentrations were measured at the end of the 6 weeks. Swimming rats had greater BMC and bone area changes at the femur and lumbar spine (p < 0.05) than the running rats and a greater whole body BMC and bone area to that of control rats (p < 0.05). There were no differences in bone gain between running and sedentary control rats. There was no significant difference in serum or bone calcium or PTH concentrations between the groups of rats. A swimming intervention is able to produce greater beneficial effects on the rat skeleton than no exercise at all, suggesting that the strains associated with swimming may engender a unique mechanical load on the bone.

Key words: Weight-bearing exercise, swimming, treadmill, DXA, bone mass, rats.

Introduction

Osteoporosis is a disease characterised by a low bone density and deterioration of the structural quality of bone, leading to weakness and fragility, which results in an increased risk of fracture (Kanis et al., 1994). An important determinant in the risk of developing osteoporosis is the peak bone mass attained during childhood and adolescence (Cadogen et al., 1998; Heaney et al., 2000). An enhanced peak bone mass can be attained through physical exercise, specifically during the growing years, as the skeleton is most responsive to exercise during this period of life (Davies et al., 2005). Attaining as maximal a peak bone mass as possible will be helpful in offsetting later development of osteoporosis and bone fragility (Burrows, 2007). Therefore, studies investigating the influence of different exercise modes on bone mineral properties, and the establishment of good animal models investigating the effects of exercise on bone, are of great clinical importance and essential in our understanding of the development of osteoporosis in humans.

Currently, the accepted model of bone development (Frost 2003; Rauch and Schoenau 2001) proposes that bone cell action is coordinated by the mechanical requirements of bone, such that when mechanical challenges exceed a set point, bone tissue is added to the location where it is mechanically necessary. Conversely, when the skeleton is subjected to loads less than habitual skeletal loads, a decrease in osteoblastic activity and an increase in osteoclastic activity occurs resulting in a decrease in bone mass (Bourrin et al., 1994; Branca, 1999).

Treadmill running stimulates bone formation and suppresses bone resorption in the rat skeleton (Iwamoto et al. 2004). This weight-bearing form of exercise has proven to have beneficial effects on the bone of both young and old rats (Bennell et al. 2002; Raab et al. 1990). These effects include increases in bone mass, bone mineral density and bone area, especially at weight bearing sites such as the femur and tibia (Holy and Zerath, 2000; Iwamoto et al., 2004; Mathey et al., 2002; Raab et al., 1990; Ward et al., 2004). Adverse effects such as reduced bone mineral density (BMD), trabecular thinning and retarded longitudinal bone growth have also been shown with treadmill running (11 weeks of training) (Bourrin et al., 1994). In addition, there are gender related differences in the bone response to treadmill running as shown by Jarvinen et al. (2003) who demonstrated that female rat bones show less of an osteogenic response to increased loading in comparison to male rats. Jarvinen et al. (2003) propose that the skeleton of a growing male rat has an increased propensity to respond to mechanical loading. There are few data pertaining to the effects of both weight bearing and non weight bearing activity in a group of young, growing, and healthy female rats as most studies have examined older and ovariectomised rats.

Studies which have examined the effects of disuse and immobilization on bone have shown the importance of mechanical load for optimal bone health (Duncan and Turner 1995, Gross et al. 2002,). In human studies, there has not been much support for the role of non weight bearing exercise to increase bone mass, yet surprisingly a number of animal studies have shown beneficial effects of swimming to the rat skeleton. In particular, a greater BMD (Hart et al., 2001), enhanced growth plate activity (Nyska et al., 1995), increases in bone mass (Huang et al., 2003), and bone mineralization (Swiss-Sivan et al., 1990) have been shown to occur in rats that have undergone swimming training. However, rats which have undergone intensive swimming training programs have also been shown not to exhibit an osteogenic response to the exercise (Bourrin et al., 1992).

Few studies comparing the concomitant effects of weight-bearing and non-weight bearing exercise on bone exist (Huang et al., 2003; Synder et al., 1992). Additionally there have been large variations in study design, exercise protocol and size and age of rats studied. An important consideration of the influence of body size on DXA data has also not been taken into account in prior studies. Also, the effects of voluntary running activity (over and above an exercise intervention) have not been studied. Therefore, the objectives of our study were to assess the effects of weight-bearing (treadmill running) and non-weight bearing (swimming) exercise on bone mineral properties and bone metabolism in rats in comparison with a group of sedentary control rats. An additional aim was to measure voluntary running activity in rats over and above their exercise intervention.

Methods

Forty growing, female Sprague Dawley rats, weighing 70-90g at the beginning of the experiment, were assigned randomly to three groups: sedentary controls (n=10), swimmers (n = 15) and runners (n = 15). Rats were housed three per cage and had free access to standard rat chow and water. We kept the rats in a temperaturecontrolled environment at 21-22 °C on a 12:12 hour light:dark cycle (lights on at 07:00). The Animal Ethics Screening Committee of the University of the Witwatersrand, South Africa approved the experimental procedures (clearance certificate number 2005/33/4).

Exercise protocol

The two groups of exercising rats underwent a two week training period, each morning (8:00-10:00), five days per week. Running rats began the training period running on a flatbed treadmill (Harvard Apparatus, USA) at 0.19 m·s⁻¹ for 10 minutes. We steadily increased the rat's time on the treadmill and speed of the treadmill so that at the end of the two weeks of treadmill running, the rats were running at 0.33 m·s⁻¹ for 30 minutes. The incline of the treadmill at 15 degrees was kept constant throughout the study period.

The rats allocated to swimming swam in a round swimming bath (water temperature of 25°C), with plastic swimming lanes to prevent the rats climbing on each other during swimming. Rats began swimming for 10 minutes, slowly increasing the time spent in the water bath throughout the two week training period, so that at the end of the two weeks, the rats were swimming for 30 minutes. After completion of the swim, the rats were dried with towels and warmed using an element heater. At the end of the two week training period eight runners were assigned to the running group and 11 swimmers were assigned to the swimming group. The rats were selected for each exercise group based on their ability to consistently complete 30min of exercise in the last week of the training period.

After selection, rats began a six week exercise programme, exercising for 30min per day, five days a week, for six weeks. The runners ran on the treadmill at 0.33m.s⁻¹ and the swimmers swam in a round swimming

bath with lanes. Sedentary control animals were placed in an empty basin filled with sawdust for the same length of time as that of the exercising animals.

Measurement of voluntary running activity was used to account for exercise over and above that of the forced exercise. During the third week of the exercise intervention, 15 rats; five sedentary controls, five runners and five swimmers were randomly selected and placed in cages (one rat per cage) with attached running wheels (circumference 1.06m). Counters (cylcocomputer, Cat eye Velo 2, CC-VL 200, CAT EYE Co., Ltd, Osaka, Japan) with a sensor (No. 169-9771, CAT EYE Co., Ltd, Osaka, Japan) were placed on each running wheel and 24h running distances (km) were recorded for one week. A complete revolution of the running wheel would be recorded when a magnet (No. 166-5120, CAT EYE Co., Ltd., Osaka, Japan) positioned on the edge of the running wheel passed under the sensor. Running wheels were allowed to rotate in one direction only. On completion of the week of voluntary running activity measurements, the animals were returned to their original cages that had no running wheels attached.

Dual energy X-ray absorptiometry (DXA)

We performed baseline and post intervention DXA scans (hologic Delphi 4500, fan beam, Bedford, USA) on all rats. Measurement and analysis was completed according to the manufacturer's protocol and all scans were performed by the same DXA technician. During the course of the study, coefficients of variation for BMC and BMD were 0.48 and 0.35%, respectively. The rats were anaesthetized with 1.5 ml·kg⁻¹ of one part ketamine hydrochloride (Anaket-V, Bayer (Pty) Ltd, South Africa) to one part medetomidine (Domitor, Novartis South Africa (Pty) Ltd) injected intraperitoneally. Each scan took approximately 15min, after which the anaesthesia was reversed with an intramuscular injection of 2 ml·kg⁻¹ atipamezole (Antisedan, Novartis South Africa (Pty) Ltd). All rats were given two days to recover following the initial DXA scan and before beginning the six week exercise programme.

We recorded the height of each rat (from scanning surface to highest point of the rat spine) and length (snout to base of tail) to correct for the magnification error associated with fan beam DXA (Blake et al. 1993). The mass of each rat was recorded to correct for the influence of body size on bone mineral content, a limitation associated with the DXA technique (Khan et al 2001). Each rat was positioned in a reproducible way for all DXA scans, on the same surface, to avoid error when comparing data. Baseline and post intervention bone area (cm²), bone mineral content (g) and bone mineral density (g·cm²) for the femur, lumbar spine and whole body was recorded. The femur and lumbar spine were scanned at high resolution, while the whole body was scanned using array mode.

Bone and blood measurements

Following the second DXA scan, conducted at the end of the six week exercise intervention, cardiac punctures were performed, while the animals were under anaesthesia. Blood was placed into two sterile tubes, with one containing EDTA, centrifuged at 4 °C, at 2000g for 10min. The serum and plasma were removed and stored at -80 °C until further analysis. All rats were euthanased by intracardiac injection of 1ml sodium-pentobarbital (Ethuanase, Kyron, Johannesburg, South Africa).

Serum ionized calcium concentration was measured using an Easylyte analyzer (Medical, Bedford, Massachusetts, USA). Plasma parathyroid hormone concentration was measured using a rat specific immunoradiometric assay (Immunotopics, CA, USA). The sensitivity of the parathyroid hormone assay was 1.0 pg·ml⁻¹.

In all animals, the femurs were dissected and wet weight (g) of each femur was recorded. Femurs were dried at 50 °C in a drying oven (Labcon 1028, LABEX, Orange Grove, South Africa) over a period of one week until weight remained constant. Dry weight (g) of each femur was recorded. Bone measurements from the cleaned, dry, right femurs of all rats were measured using digital calipers (Harbor Freight Digital Caliper, SKU 47257, Harbor Freight Tools, USA). Femur length (mm) was measured from the head of the femur to the medial epicondyle and femur diameter (mm) was measured at the midpoint of the bone along the shaft in each rat. The right femur was then placed in an ashing oven. The ash of each femur was mixed with 1ml hydrochloric acid (10%) and bone calcium concentration measured with an Easylyte analyzer (Medical, Bedford, Massachusetts, USA).

Statistical analysis

The percentage change in bone mineral content (g), bone mineral density (g·cm⁻²) and bone area (cm²) after the six week intervention was calculated and recorded for each rat. An analysis of covariance (ANCOVA) controlling for rat mass with Tukey *post hoc* test was used for analysis of percentage bone mineral content, percentage bone mineral density and percentage bone area for all sites analyzed. All other variables were analysed using analysis of covariance (ANCOVA) with Tukey *post hoc* test between the three groups of rats, except for parathyroid hormone measurements which were analysed using a Kruskal Wallis non parametric test. Values are represented as mean \pm standard deviation and p < 0.05 was considered significant.

Results

Anthropometric data

There was no significant difference (F=2.229, p = 0.127) in the initial mass of the three groups of rats or the mass at the end of the study. All rats grew at approximately equal rates over the study period and no significant differences in weight gain were observed (Figure 1). Initial length and height as well as post intervention length and height measurements were also not significantly different (F=0.505, p = 0.61) between the three groups for the two time measures.

Dual energy X-ray absorptiometry

Baseline bone mineral content, density and area at the femur, lumbar spine and whole body (adjusted for body mass) for the three groups of rats are presented in Table 1. There were no significant differences in bone mass at any

site or between any of the groups before the intervention.



Figure 1. Average weekly weight measurements of rats within each study group (runners= \blacksquare , swimmers= \bullet and control group \triangledown). Arrow indicates the start of the exercise intervention.

Whole body: Figure 2 shows the percentage change from pre-exercise in whole body bone mineral content (BMC), bone mineral density (BMD) and bone area in rats that ran, swam or were sedentary for six weeks. The percentage change in BMC was significantly higher in the swimming group than in the control (F=2.36, p < 0.001) and running groups (F=3.82, p < 0.05). The percentage change in BMD was significantly higher in the swimming group (F=3.62, p < 0.001) compared to runners, but not the controls. The percentage change in bone area for the whole body was significantly higher in the swimming group (F=8.12, p < 0.001) compared to that of the controls.

Table 1. Bone mineral content, bone mineral density and bone area measured at the femur, lumbar spine and whole body, for the three groups, before a 6wk exercise intervention. Data are means (±SD).

	Bone mineral	Bone mineral	Bone area
	content (mg)	density (mg·cm ⁻²)	(cm ²)
Femur			
Control	164 (31)	174 (17)	.94 (.09)
Swimmers	146 (22)	169 (12)	.86 (.07)
Runners	178 (14)	180 (7)	.99 (.05)
Lumbar spine			
Control	301 (50)	160 (13)	1.87 (.16)
Swimmers	279 (38)	157 (10)	1.77 (.15)
Runners	339 (36)	168 (9)	2.01 (.12)
Whole bod	у		
Control	4497 (558)	111 (9)	40.54 (2.77)
Swimmers	4106 (371)	108 (6)	38.05 (2.10)
Runners	4681 (351)	118 (49	39.77 (2.74)

Lumbar spine: Figure 3 shows the percentage change from pre-exercise in lumbar spine bone mineral content (BMC), bone mineral density (BMD) and bone area in rats that ran, swam or were sedentary for six weeks. The percentage change in BMC at the lumbar spine was higher in the swimming group of rats, than in the runners (F=3.58, p < 0.05). There was no significant difference in the percentage change in BMD between the three groups of rats (F=1.98, p = 0.16). The percentage change in bone area was significantly lower in the run-

ning group compared to that of the swimming group (F=4.29, p < 0.001).



Figure 2. Percentage change (%) in bone mineral content (BMC), bone mineral density (BMD) and bone area of the whole body of the rat for each exercise group. Bars represent means \pm standard deviation. * p < 0.05; ** p < 0.001.

Femur: Figure 4 shows the percentage change from pre-exercise in femur bone mineral content (BMC), bone mineral density (BMD) and bone area in rats that ran, swam or were sedentary for six weeks. The percentage change in BMC was significantly greater in the swimming group than in runners (F=3.41, p < 0.001). There was no significant difference in the percentage change in BMD at the femur between the three groups of rats (F=1.52, p = 0.24). The percentage change in bone area at the femur was significantly different between the three groups of rats with the swimming rats greater than that of the running rats (F=4.41, p < 0.05).



Figure 3. Percentage change (%) in bone mineral content (BMC), bone mineral density (BMD) and bone area of the lumbar spine for each exercise group of rats. Bars represent means \pm standard deviation. * p < 0.05; ** p < 0.001.

Femur length and diameter

There was no significant difference for femur length between the runners $(34.55 \pm 1.40\text{mm})$, swimmers $(33.50 \pm 0.70\text{mm})$ or sedentary control rats $(34.21 \pm 1.99\text{mm})$, F=1.34, p = 0.28). The swimming group of rats had the shortest femurs, however exhibited the greatest femur diameter $(4.33 \pm 0.23\text{mm})$ compared to the runners $(4.20 \pm 0.27\text{mm})$ and controls $(4.32 \pm 0.36\text{mm})$, but there was no significant difference in femoral diameter between the three groups (F=0.54, p = 0.59).

Evening running activity

Figure 5 shows the average running distances (km) measured over a period of one week for the running group of rats compared to swimmers and control rats on the voluntary running wheels. The swimming group of rats recorded the lowest average running distance, which was significantly less than the control rats (F=18.57, p = 0.012) and the running rats (p < 0.001).



Figure 4. Percentage change (%) in bone mineral content (BMC), bone mineral density (BMD) and bone area of the femur for each exercise group of rats. Bars represent means \pm standard deviation. * p < 0.05; ** p < 0.001.

Serum and bone ionized calcium and PTH concentration

There was no significant difference in serum ionized calcium concentrations between the three groups of rats (F=0.01, p = 0.98) where the serum ionized calcium concentration in the control rats was $0.99 \pm 0.16 \text{ mmol}\cdot\text{I}^{-1}$, the swimming rats was $0.98 \pm 0.85 \text{ mmol}\cdot\text{I}^{-1}$ and the running rats was $0.94 \pm 0.77 \text{ mmol}\cdot\text{I}^{-1}$. Ionized calcium in the bone was not significantly different between the three groups with the control group having $14.0 \pm 1.3 \text{ mmol}\cdot\text{I}^{-1}$ and the swimmers $14.5 \pm 1.3 \text{ mmol}\cdot\text{I}^{-1}$ (F=0.33, p = 0.72).



Figure 5. Evening running distance (km) for each exercise group recorded over a period of one week. Bars represent means \pm standard deviation. * p < 0.05.

Plasma PTH concentrations after a six week exercise programme of running, swimming and a six week sedentary programme were not significantly different between the three groups (F=0.27, p = 0.77). The PTH concentrations were as follows: control rats (26.9 ± 6.7), runners (22.3 ± 6.61) and swimmers (23.8 ± 9.2 pg·ml⁻¹).

Discussion

Our study has shown that in growing female rats, swimming is more effective for enhancing bone mineral content and area at the whole body than not exercising at all. The sedentary control rats showed increases in bone mineral content, density and area associated with normal growth that were no different to bone mass gains exhibited by runners. In addition, our study showed that rats assigned to the swimming group voluntarily ran almost half the distance at night compared with the running group of rats. While control rats voluntarily ran a similar distance at night to runners, they were not undergoing the additional exercise regime during the day. Bone mass gain was lowest in the running group, inferring that increases in bone mineral content, area and density may have been stunted as a result of the exercise protocol administered in combination with the rat's high voluntary activity at night, or that a running exercise intervention of equal duration to swimming may not be intense enough to elicit an osteogenic effect. Our rats did not have significantly different gains in body mass throughout the study, indicating that each group was undergoing much the same growth rate. Therefore, the exercise influenced bone in each group at approximately the same growth phase.

Two studies have compared the effects of swimming and running in young growing rats (Huang et al., 2003; Synder et al., 1992), both reporting an increase in femur and tibia biomechanical properties in swimmers than in runners. Although Huang et al (2003) did not report significantly greater BMD in swimming rats versus runners; they also did not take into account the effects of body size difference in their DXA analysis. In our study, a running intervention of equal duration to a swimming intervention (but not necessarily of equal intensity) appears to be no better for enhancing bone mass acquisition than doing no additional exercise at all.

Swimming exercise in ovariectomized rats also has been shown to produce greater bone mineral density, mechanical properties and histomorphometric indices in the femur compared to that of sedentary controls (Hart et al., 2001). To date, only Warner et al. (2006) have matched the mechanical load associated with running to that of a swimming protocol. Warner's study (2006) reported that a swimming protocol of equal mechanical intensity to a running protocol stimulated osteogenic adaptations on the humerus and femur which are different to those brought about by normal cage activity and greater than those afforded by running activity. We did not measure intensity of exercise in our study, and therefore speculate our running intervention was either not long enough or intense enough to bring about an osteogenic response.

Although rats are active in the dark phase, the exercise protocols used in most studies are done in the light phase, without measuring whether voluntary activity in the dark phase is altered. In our study, evening running activity recordings were taken to establish whether the control rats were compensating at night (the time when animals are normally active) for their lack of exercise during the day. Despite runners exercising five days a week, they still recorded the greatest evening running distances. Swimmers ran the least distance during the night with control rats having greater evening running recordings than swimmers. It appears that treadmill running during the day did not attenuate the rat's propensity for voluntarily running as evidenced by greater running distances at night. To our knowledge, no other study examining the effects of exercise on bone gain in rats has taken voluntary running activity into account. Whether a swimming regimen suppresses night time voluntary running activity remains to be determined.

A study conflicting with our results investigated the effect of five weeks of swimming on rat bone and documented adverse effects of swimming. Bone loss was seen to occur in the femur of swimming rats (Bourrin et al., 1992). The duration of the intervention period was similar to our study, however by the end of the study rats were swimming for six hours per day, a duration which could have resulted in detrimental overtraining effects.

Load-bearing exercise resulting in an increased skeletal mass is a well known phenomenon. Bone formation especially at weight bearing sites, suppression of bone resorption, increases in bone mass and increases in bone mineral content of the femur and tibia have been documented with treadmill running (Iwamoto et al., 2004). Treadmill running also has been found to increase bone mineral density, cortical bone area, bone stiffness and have an effect on bone morphometry in rat femurs, tibia and vertebrae (Wheeler et al., 1995). It is important to note that in vitro evidence that excessive high-impact exercise potentially induces joint inflammation and degradation has also been shown (Sun et al., 2004). This is an important consideration in the prescription of osteogenic exercises. We have shown that a thirty minute running intervention lasting five days a week for six weeks is not effective for enhancing bone mass gains in growing female rats and results in similar growth associated bone gains exhibited by the control rats. However, the results

of our study do suggest that a moderate swimming protocol may elicit a more favourable bone mass response to that of not exercising at all.

Previous studies document that bone growth as a result of exercise results in an increased demand for minerals. This demand is satisfied by an increase in serum 1.25 dihydroxyvitamin D3 levels and increased intestinal absorption of calcium (Iwamoto et al., 2004; Yeh and Aloia, 1990). Therefore, this increase in absorption would result in an increased plasma calcium concentration with exercise. However, our study did not document significant differences in plasma or bone calcium or PTH concentrations between the groups of rats. It is possible that the combination of anaesthetic drugs that we used masked any differences in PTH concentrations that may have existed. Previous studies have shown that certain anesthetic agents, specifically a combination of ketamine plus xylazine result in a marked increase in serum PTH concentrations in rats (Mallya et al. 2007; Schultz et al. 1995).

In our study, we did not compare the intensity of the swimming to that of the running, but rather kept the duration of the exercise constant. Measuring heart rate or oxygen consumption of each rat would provide a means in which to control for the effect of exercise intensity and therefore eliminate conditions such as overtraining, which may have occurred in the running rats. Alternatively, the intensity of the running regime may not have been intense enough to obtain greater bone mass gains than those associated with normal growth.

Conclusion

In conclusion, a six week swimming exercise intervention resulted in substantial increases in bone mineral content and area in growing female rats. The effects of swimming on rat bone were greater than those observed in rats in the sedentary group. Additionally, we have shown that rats that undergo a treadmill exercise regime voluntarily run, in addition to the treadmill running, almost twice the distance compared with that of the swimmers. Our study demonstrates the potential of swimming as a means of increasing the attainment of a high peak bone mass during growth, an important factor for decreasing the risk of developing osteoporosis in humans. A swimming intervention of six weeks is able to produce substantial, beneficial effects on the rat skeleton. While it is widely accepted that weight-bearing exercise results in positive effects on bone, our study shows that swimming, a nonweight bearing exercise provides an important potential alternative, which warrants further investigation, to weight-bearing exercise in humans.

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Key points

- A six week swimming intervention is able to produce greater osteogenic effects on the rat skeleton than no exercise.
- A daily treadmill running intervention does not attenuate a rats propensity to run voluntarily at night.

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