J Musculoskel Neuron Interact 2003; 3(1):47-52

Original Article

Hylonome

Progressive isometric force training and bone mass in rats

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Abstract

In ten growing male Wistar rats, isometric strength training for 69 days (3-6 times vertical gripping position on a wire-netting during 2 x 30 s, with progressive loading of the tail through a 50-200 g indwelling clip), fat mass and plasma leptin concentrations were lower on day 70 than in ten sedentary controls. Muscle mass and femoral trabecular and cortical bone mineral density were simultaneously higher in exercised animals than in controls. Such an effect might result from decreased bone resorption. At the end of the training period no difference concerning plasma osteocalcin concentration was observed between exercised and resting rats while urinary deoxypyridinoline excretion was lower in the former than in the latter.

Keywords: Isometric Strength Training, Bone, Osteocalcin, Deoxypyridinoline, Rat

Introduction

Symptoms of osteoporosis do not generally occur until after menopause although bone loss starts much earlier in life[1,2]. Hormonal status, especially estrogen or androgen availability in women and men, respectively, plays a major role in preventing bone loss[3,4]. Recent studies estimate that as much as 70% of peak bone mass is genetically determined[5-7]. Two other factors which can be controlled, nutrition[8] and exercise[9], also greatly affect peak bone mass. Exercise, such as resistance training or weight-bearing activities, like running or walking, have an osteogenic effect on developing and maintaining bone mineral density (BMD)[10-15]. Femur and humerus from both young and senescent trained rats have a greater resistance to fracture than those from weight-matched sedentary controls[16,17].

However the optimal training mode has not been published. Artificial loading imposed by external stimulus on the bone demonstrates that dynamic loading is more effective than static one[18,19]. Intermittent hydrostatic compression (IHC) increases bone formation in cultured fetal mouse calvariae compared to non-stimulated cultures. Treatment with IHC promotes the osteoblastic phenotype in bone organ culture and in osteoblasts[20]. Using the rat tibia 4-point bending model[21], Robling et al.[22] compared the osteogenic effect of a 54 N force delivered in 1, 2, 4 or 6 bouts on each of 3 loading days. They show that 360 daily loading cycles applied at intervals of 60 x 6 or 90 x 4 represent a more osteogenic stimulus than 360 cycles applied all at once, and that mechanical loading is more osteogenic when divided into discrete loading bouts. Yao et al. developed a cage that forces the rat to raise up on its hindlimbs during feeding or drinking for 12 weeks. Making intact rats rise to erect bipedal stance increased muscle mass, cortical bone volume and periosteal bone formation. If animals were orchidectomized, the bipedal stance feeding partially prevented cancellous bone loss in the proximal tibia metaphysis, and it totally prevented net bone loss in the tibial shaft by inducing periosteal bone formation[23].

The word «isometric» is defined as «iso» – equal of the same – and «metric» length. Isometric, as it pertains to muscle training, involves tensing muscles against other muscles or against an immovable object while the length of the muscles remains unchanged. Effective isometric training is achieved when the muscle tension is maintained over a certain period of time. Thus, isometric training is best defined as the sustained contraction of a muscle over a certain period of time. Isometric contractions represent a significant part in some sport practice (gymnastics, climbing, skiing, weight-lifting, body building, pistol shooting). Consequently, it often constitutes a part of training in non-specific preparation of athletes. Training or rehabilitation of some muscle groups using electrostimulation also consists of isometric contractions. However, because of the high constraints generated by isometric training which makes it difficult to accept over a long period, and al-

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Accepted 28 June 2002

Weeks	Number of series	Loading (g)
1	**3**x(2x30s)	**50**
2	**4**x(2x30s)	**50**
3	**4**x(2x30s)	**100**
4	**5**x(2x30s)	**100**
5	**5**x(2x30s)	**150**
6	**6**x(2x30s)	**150**
7	**6**x(2x30s)	**180**
8	**7**x(2x30s)	**180**
9	**7**x(2x30s)	**200**
10	**8**x(2x30s)	**200**

Table 1. Training program.

so because man is a biped and active throughout the day, he exerts his legs' muscles. Therefore, in man, it is difficult to obtain scientific information about the results of training only proceeding from isometric contractions[24]. A program of resistance training based on a series of isometric contractions by using a model of «climbing» rat with additive loads has already been presented[25]. In the work reported here, we observed the influence of this training program for 10 weeks on muscle and bone mass in intact male rats.

Materials and methods

Animals and treatment

This experiment was carried out in accordance with current legislation on animal experiments in France. Twenty male Wistar rats weighing 296 ± 6g were used at 2.5 months of age. Ten rats were randomly assigned to an exercise group (E). The others were used as controls (C). Each rat was housed in a 22 x 22 x 18cm plastic cage, allowing separation and collection of urine and faeces, at 22 ± 1ºC, with a 12h : 12h light-dark cycle. The animals were fed ad libitum a laboratory chow (UAR, Villemoisson sur Orge, France) containing 16% protein, 3% fat, 0. 8% calcium and 0. 6% phosphorus. The daily consumption of each rat was measured and each animal was weighed once per week. The urine of each rat was collected during a 24 hour period on day 68.

Each exercised rat (E) was trained every morning, six days per week for 10 weeks, according to an already described protocol[25]. Briefly, in order to obtain a 100% isometric model of training, each rat was set on the horizontal floor of a box and then the box was put in a vertical position. The floor being made with a wire-netting, the animal gripped with its claws and remained in the climbing position. This occurred 3-6 times for 2 x 30-second periods separated by resting for 20 seconds. Each animal was allowed to rest for 3 minutes between each gripping period. The training programme progressively increased in intensity by adding a load to the tail of the rat by means of an indwelling clip weighing 50-200 g (Table 1).

On day 70, the rats were killed by cervical dislocation. Blood was collected by cardiac puncture. After centrifugation, plasma was harvested and frozen until analysis, which was performed during the 2 weeks following slaughter. Femurs were separated from adjacent tissue, cleaned and used for physical measurements.

Physical measurements

Body composition and femoral bone density. On day 67, under light chloral anaesthesia, lean and fat mass were measured on each animal by dual X-ray absorptiometry (DEXA)[26], using a Hologic QDR 4500A X-ray densitometer (Hologic, Massy, France). On day 71, total right femoral BMD (T-BMD) was also determined by DEXA. Furthermore, the BMDs of two subregions, one corresponding to the distal metaphyseal zone (M-BMD), which is rich in cancellous bone, and the other to diaphyseal zone (D-BMD), mainly cortical bone, were assessed[27]. These two subregions correspond to the middle third and the distal third of the femoral bone, respectively[28].

Strength measurement. On day 70, just before slaughter under light chloral anaesthesia, a longitudinal incision of the right posterior hindlimb permitted access to the sciatic nerve which was isolated and charged on an electrode for stimulation. The Achilles tendon was isolated, ligated and the calcaneus was cut. Then the rat was set in dorsal decubitus, its right knee was fixed to the table with a needle and the tendon was linked to the force transducer (F621 TC ; 10 daN capacity ; 0. 01 N resolution, calibrated with known weights) connected to software (Bio 1000, Nortek, Villeneuve d'Ascq, France). For twitch contractions, the sampling frequency was 2000 Hz. For tetanic contractions , it was 40 Hz. The muscle was extended with a preload of 0.2 N. Then the maximal strength recorded corresponded to the capacity of the extended muscle, slightly lower than the maximal tetanic force[29]. Measures were recorded on a computer. Contraction time (from baseline to peak) per twitch contractions was automatically recorded by the software.

Femoral mechanical testing. Immediately after collection, each left femoral bone was placed in 0.9% NaCl at 4°C. Mechanical femoral resistance was determined 24 hours later by a three-point bending test. Each bone was secured on the two lower supports of the anvil of a Universal Testing Machine (Instron 4501, Instron, Canton, MA, USA). The upper roller diameter was 6 mm. The crosshead speed for all the tests was 0.5 mm / minute. The load at rupture was determined automatically by the Instron 4501 software. To ensure comparable test sites, the femur was always mounted so that the crosshead was applied in the middle of the shaft of the bone. Using 450-g rats, the span of the specimen that was loaded was 20 mm to guarantee that 85-90% flexure of the bone was caused by bending. This test had been previously validated by using Plexiglas standard probes[30]. Results are expressed in newtons.

In ashed bone, calcium was measured by atomic absorption spectrophotometry.

	C	E
Fat mass	15.4±3.3	11.±11.9*
Lean mass	81.9±6.0	86±5.7*
T-BMD	0.2511±0.0054	0.2752±0.0137**
D-BMD	0.2357±0.0080	0.2510±0.0129*
M-BMD	0.2584±0.0075	0.2857±0.0147**
Ca content	545±17	598±37*

Table 2. Fat and lean mass (% from body weight), total (T-BMD), diaphyseal (D-BMD) and distal metaphyseal (M-BMD) femoral bone density ($g.cm^{-2}$) and total femoral calcium (Ca) content (g) in exercised (E) and control (C) rats (means±SEM). *p<0.05 vs C; **p<0.01vs C.

Biochemical analysis

Marker for osteoblastic activity. Plasma osteocalcin (OC) concentration was measured by homologous radioimmunoassay using rat OC standard, goat anti-rat OC antibody; 125-I labeled rat OC, and donkey anti-goat second antibody (Biochemical Technologies Kit, Stoughton, MA, USA). The lowest limit of detection for this assay was 55 pg / mL, and the intra- and inter-assay variations were 7% and 9%, respectively.

Marker for bone resorption. Deoxypyridinoline (DPD) in urine was measured by radioimmunoassay (RIA) (Pyrilinks D kit, Metra Biosystems, Mountain View, CA, USA). The assay required the addition of 50 mL of urine sample (or DPD standard or control) to each well of the DPD-coated microplate. The monoclonal antibody against DPD was added to the plate, the free DPD in urine competing with the DPD coated on the plate for the antibody. A second antibody conjugated to alkaline phosphatase was added to the plate to bind with antibody against DPD. A substrate p-nitrophenylphosphate was added to produce a yellow color. Optical density was measured at 405 nm. The lowest limit of detection for the assay was 2 nmol. The intra- and inter-assay variation was 5% and 7%, respectively. Results are expressed as nanomolar DPD/ mM creatinine to avoid the possible influence of glomerular filtration rate[31]. The creatinine assay was a modified Jaffé's method in which picric acid forms a yellow compound with creatinine.

RIA for plasma leptin concentration used an homologous assay incorporating anti-rat leptin antibody and rat leptin as the standard (Rat Leptin RIA Kit, Linco Research Inc., St Charles, MO, USA). In our experimental conditions, the lowest limit of sensitivity was 0.5 ng / mL, and the intra- and inter-assay variations were 1.5% and 2.5%, respectively.

Statistics. Results are presented as mean ± SEM. The Mann-Whitney Wilcoxon U-test was used to compare differences between groups.

Results

During the whole experimental period, body weight (BW) increase was lower in E (from 292 ± 12 to 421 ± 21, i.e., + 44 ± 2% ; P<0. 01 vs. C) than in C (from 299 ± 5 to 467 ± 22 g, i.e., + 56 ± 3%). From the second to the tenth week of this training period, the mean daily food intake was slightly lower in E (27 ± 1 g) than in C (29 ± 2 g ; P<0. 05).

On day 67, fat mass was lower in E than in C, while lean mass was higher in E than in C (Table 2). Maximal isometric strength (N) was 61% greater in E (34 ± 8) than in C (21 ± 7 ; P<0. 01). Although femoral failure load (N) was not different in E (118 ± 4) and in C (106 ± 16), the training program significantly increased T-BMD, D-BMD and M-BMD (Table 2).

No difference concerning plasma osteocalcin levels (ng / mL) was observed between E (35. 2 ± 3. 4) and C (38. 9 ± 4. 4), while urinary DPD excretion (nmol DPD / mmol creatinine) was lower in E (114 ± 9) than in C (130 ± 3 ; P<0. 05).

Plasma leptin concentration (ng / mL) was twice lower in E (1. 4 ± 0. 5 ; P<0. 05) than in C (3 ± 1; P<0. 05). A positive linear relationship was observed between fat mass (y) and plasma leptin concentration (x) : y = 4. 57 x + 34. 96 ; r = 0. 587 ; P<0. 05).

Discussion

Each rat was separately housed in a small cage; C can be considered as sedentary rats. Thus, although unmeasured, their energy expenditure during the experimental period was probably much lower than in E. This, together with the daily food consumption slightly lower in E than in C would explain the lower fat mass in E than in C. The greater lean mass measured in E would partly explain the 61% increase in isometric force following the training program.

Treadmill running[17,32], jumping[33], swimming[34], climbing ladders[35], voluntary wheel running in cages[36], overloading one limb by immobilizing the other[37], making the rat rise to erect bipedal stance for feeding or drinking[23] has been shown to prevent castration-induced bone loss and/or to increase bone mass in rats. To our knowledge, our work is the first which demonstrates that progressive isometric strength training is also able to increase muscle mass and both trabecular and cortical femoral BMD in growing rats (Table 2). Such an in-

crease in femoral BMD was not associated with increased femoral failure strain. Bone tissue is able to adapt its mass[38,39], architecture[40] and mechanical properties[41] to mechanical loading environment. Although the mechanical strength of bone partly depends on its microstructure[42], it is generally accepted that it depends on both bone mass and bone quality[43,44]. This would indicate that in our experimental conditions where bone quality was not measured, the increase in bone mass (Table 2) was not sufficient to induce a higher femoral failure load.

The general effects of exercise on bone are strain-related increases in bone formation and decreases in bone resorption, which results in increased bone mass[45]. The forces applied to bone result from muscular contraction (as opposed to impact loading) and they are transmitted by tendon insertions on the periosteal surface of bone. This explains why a jumping exercise increases periosteal, but not endosteal bone formation and breaking strength in murine femora and tibiae[33]. In our experimental conditions, plasma OC concentration, a marker for osteoblastic activity[46], was not different in E and C. However urinary DPD excretion was lower in E than in C. This indicates that isometric strength training might increase bone mass mainly by inhibiting bone resorption[31,45]. Such an effect occurred as well on trabecular bone than on cortical bone since both M-BMD (trabecular bone) and D-BMD (cortical bone)[27,28] were higher in E than in C (Table 2). Although the regions of metaphysis are rich in cancellous bone as compared to other regions but still have cortical bone over 30%, the data of the M-BMD do not strictly represent trabecular bone. Particularly, the muscular contraction forces applied to periosteal surface of metaphysis. Thus, periosteal bone formation in this region (cortical bone) was probably also increased.

At the end of the experimental period, plasma leptin concentration was twice lower in E than in C. Leptin is mainly secreted by adipocytes[47]. However, the influence of exercise on leptin is more or less controversial. In obese human male subjects, endurance exercise training decreased plasma leptin independently of body fat percentage[48]. In healthy endurance-trained young males, a positive linear relationship was observed between fat mass and plasma leptin concentration[49]. In the same way, in our animals, the highest plasma leptin concentration was measured in C which possessed the highest fat mass. The influence of leptin on bone metabolism is still poorly understood. According to Ducy et al.[50], leptin would be a potent inhibitor of bone formation acting through the central nervous system. This would explain the increased bone mass in db/db (leptin resistant) mice. However in obese ob/ob (leptin deficient) mice, recombinant murine leptin (50 mg / mouse / day for 3 weeks) increased femoral length, total bone area, bone mineral content and BMD 4[51]. Thus, our results do not allow us to decide the way by which strength training increased bone mass. Neither do they allow us to know whether this type of exercise would be effective in older, slower growing rats. Nevertheless, in 15-month-old male Wistar rats, moderate treadmill-running for 90 days prevented an age-related decrease in femoral BMD. This effect was also associated with a decrease in urinary DPD excretion without change in plasma OC concentration at the end of the experimental period[52]. Maddalozzo and Snow compared the effects of a moderate intensity resistance training program (MITP) with a high intensity standing free weight exercise program (HITP) performed for 12 weeks on bone mass of healthy 55-65 year-old men and women. HITP resulted in a gain in spine BMD in men, but not in women. MITP produced no change in either gender at this site. Increases were observed at the greater trochanter in men regardless of training intensity but not in women at any hip site[53].

In conclusion, progressive isometric strength training for ten weeks increased trabecular and cortical femoral bone mass in growing rats. On day 70, this increase was associated with decreased urinary DPD excretion (a marker for bone resorption) without significant variation in plasma osteocalcin concentration, a marker for osteoblastic activity.

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