Influence of treadmill running on femoral bone in young orchidectomized rats

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Horcajada, M.-N., V. Coxam, M.-J. Davicco, N. Gaumet, P. Pastoureau, C. Leterrier, J. Culioli, and J.-P. Barlet. Influence of treadmill running on femoral bone in young orchidectomized rats. J. Appl. Physiol. 83(1): 129–133, 1997.—Forty 6-wk-old male Wistar rats weighing 308 ± 24 g were divided into two groups. On day 0, the 20 animals in one group were surgically castrated and the other group was sham operated. Within each group, 10 rats were selected for treadmill running (60% maximal \( \dot{O}_2 \) consumption, 1 h/day, 6 days/wk for 15 wk). The 20 sedentary rats were used as controls. At the time the rats were killed (day 105), running had no significant effect on femoral mechanical properties either in castrated or in sham-operated rats. Femoral bone density was lower in orchidectomized than in sham-operated rats. Nevertheless, it was higher in exercised than in sedentary rats. Femoral Ca content paralleled changes in bone density. Treadmill running had no significant effect on plasma osteocalcin concentration but inhibited the increase in urinary deoxypyridinoline excretion observed in castrated rats. Image analysis (measured at the distal femoral diaphysis) revealed that these effects mainly resulted from decreased trabecular bone resorption in castrated exercised rats.

osteoporosis; exercise; osteocalcin; deoxypyridinoline

In rats, bone remodeling imbalance spontaneously occurs with aging and after castration, because bone resorption then exceeds bone formation (4, 9). In 6-wk-old female rats, immobilization for 6 wk results in an increase in bone resorption and a rapid fall in bone formation, whereas treadmill running (20 m/min for 60 min/day for 3 wk) is associated with an initial increase and then a decrease in bone resorption, with a sustained bone formation (42). The purpose of the present experiment was to study the influence of treadmill running on bone metabolism in young castrated male rats.

METHODS

Treatment of Animals

The animal protocol for this study was reviewed and approved by the Animal Care and Use Committee of Institut National de la Recherche Agronomique Clermont-Theix. Thus 40 male Wistar rats weighing 308 ± 24 (SE) g were used at 6 wk of age. The animals were randomized by body weight in two groups. On day 0, under chloral anesthesia (8 g chloral hydrate/100 ml saline; 0.4 ml/100 g body wt ip) the animals (n = 20) from the first group were surgically castrated (CX). The 20 other rats were sham operated (SH). Seventy-two hours after surgery, 10 rats within each group were selected according to their willingness for treadmill running. These exercised (sham-operated exercised [SHE] and castrated exercised [CXE]) rats were then trained daily to run on a flatbed treadmill. During the first 2 wk, the speed of the treadmill and the duration of each running session were gradually increased from 15 m/min for 15 min to 20 m/min for 60 min, to reach 30 m/min for 60 min at the end of the experiment (day 105). Thus, during the experimental period, the running speed corresponded to ~60% of \( \dot{V}_{O_2 \text{max}} \) for these animals (13, 18, 39). Control resting (SHR and CXR) rats were handled twice daily at 1-h intervals to mimic the stress induced by handling before and after running.

Each rat was housed individually in a 22 × 22 × 18-cm plastic metabolic cage allowing separation and collection of feces and urine (Établissements Pajon, Fleury les Aubrais, France), at 21°C, with a 12:12-h light-dark cycle. The animals were fed a laboratory chow (Usine d'Alimentation Rationnel, Villemoisson/Orge, France) containing 0.84% calcium and 0.78% phosphorus. To prevent hyperphagia induced by castration, the daily consumption of each rat was measured and each animal received the mean quantity of the chow consumed by sham-operated rats (SHE and SHR) during the previous day. Each rat was weighed every Tuesday before running. Every 2 wk, immediately after the running session, urine from each animal was collected during a 24-h period to measure the excretion of deoxypyridinoline (DPD), a marker of bone resorption (23).

On day 105, rats were killed by cervical dislocation. Blood was collected by cardiac puncture.
Table 1. Influence of running and/or orchidectomy on weights of heart, lungs, and seminal vesicles and on length, diameter, and midshaft area of right femur measured at necropsy

<table>
<thead>
<tr>
<th>Group</th>
<th>Heart, g</th>
<th>Lungs, g</th>
<th>Seminal Vesicles, g</th>
<th>Femur Length, mm</th>
<th>Femur Diameter, mm</th>
<th>Femur Midshaft area, mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>CXE</td>
<td>1.54 ± 0.03†</td>
<td>2.62 ± 0.09†</td>
<td>0.100 ± 0.006*</td>
<td>36.2 ± 0.5</td>
<td>3.4 ± 0.3</td>
<td>2.04 ± 0.08</td>
</tr>
<tr>
<td>CXR</td>
<td>1.35 ± 0.04</td>
<td>2.41 ± 0.12</td>
<td>0.103 ± 0.011*</td>
<td>36.0 ± 0.3</td>
<td>3.4 ± 0.1</td>
<td>2.02 ± 0.07</td>
</tr>
<tr>
<td>SHE</td>
<td>1.75 ± 0.06†</td>
<td>2.61 ± 0.09†</td>
<td>1.728 ± 0.310†</td>
<td>36.6 ± 0.4</td>
<td>3.4 ± 0.1</td>
<td>1.86 ± 0.08</td>
</tr>
<tr>
<td>SHR</td>
<td>1.48 ± 0.05</td>
<td>2.39 ± 0.09</td>
<td>1.688 ± 0.084†</td>
<td>37.1 ± 0.4</td>
<td>3.6 ± 0.1</td>
<td>2.09 ± 0.06</td>
</tr>
</tbody>
</table>

Values are means ± SE. CXE, castrated exercising rats; CXR, castrated sedentary rats; SHE, sham-operated exercising rats; SHR, sham-operated sedentary rats. *P < 0.05 compared with SHR. †P < 0.01 compared with CXR.

After centrifugation, plasma was harvested and frozen until analysis. The success of orchidectomy was confirmed by a marked atrophy of seminal vesicles in CX animals (Table 1). The heart and the lungs of each rat were also collected and immediately weighed. Femurs were cleaned from adjacent tissue and used for physical and chemical measurements.

Physical Measurements

Femoral mechanical testing. Immediately after collection, the length (L) of the right femur and the mean diameter (d) of the femoral diaphysis were measured by using a caliper. Then each bone was kept in 0.9% NaCl at 4°C. The mechanical resistance of the femoral bone was determined 24 h later, by using a three-point bending test. Each bone was secured on the two lower supports (diameter 4 mm, length 20 mm) of the anvil of a Universal Testing Machine (Instron 4501, Instron, Canton, MA). The upper roller diameter was 6 mm. The crosshead speed for all the tests was 0.5 mm/min. The force (F) at rupture was determined, and the stress (σ) was calculated according to the following equation: σ = F L/rd³. This testing procedure had been previously validated by using Plexiglas standard probes (33). Results are expressed in megapascals.

Bone mineral density (BMD). Dual-energy X-ray absorptiometry (DEXA) measurements were made with a Hologic QDR-1000 X-ray bone densitometer (Hologic France, Massy, France). Total femoral BMD (TFBMD) was determined. Furthermore, the BMDs of two subregions, one corresponding to the metaphyseal zone (MBMD), which is rich in cancellous bone, and the other to diaphyseal zone (DBMD), which is rich in cortical bone, were measured (22).

Image analysis. To characterize static cancellous bone, frontal sections of the digital femur were cut with a saw (Isomet 2000, Buehler), ground to 80-μm sections (Metaserv 2000 polisher, Buehler), stained with Von Kossa’s reagent, and analyzed with an automated television-microscope image-analysis system, as previously described (24). Cancellous bone area and perimeter and thickness of trabeculae were determined.

Biochemical Analysis

Marker of osteoblastic activity. Plasma osteocalcin (OC) concentrations were measured by homologous radioluminometric assay by using rat OC standard, goat anti-rat OC antibody, 125I-labeled rat OC, and donkey anti-goat second antibody (Biochemical Technologies kit, Stoughton, MA). The lower limit of detection for the assay was 0.06 ng/ml, and the intra- and inter-assay variations were 6.8 and 8.9%, respectively (10).

Marker of bone resorption. DPD in urine was measured by radioimmunoenzymatic assay by using a Pyrilinks-D kit (Metra Biosystems, Mountain View, CA). The assay of DPD requires the addition of 50 μl of urine sample (or DPD standard or control) to each well of the DPD-coated microplate. The monoclonal antibody against DPD is added to the plate, and the free DPD in urine competes with the DPD coated on the plate for the antibody. A second antibody conjugated to alkaline phosphatase (goat anti-rabbit immunoglobulin G alkaline phosphatase) is added to the plate to bind with antibody against DPD. A substrate, p-nitrophenylphosphosphate, is added to produce a yellow color. Optical density was measured at 405 nm (30). In the conditions of this study, the lower limit of detection for the assay was 3 nmol. The intra- and interassay variations were 6 and 8%, respectively.

Results are expressed as nanomoles of DPD per millimole of creatinine (23). The urinary creatinine assay is used to adjust DPD values for variation in urine volume. This assay requires the addition of 50 μl of urine sample (or DPD standard or control) to each well of the DPD-coated microplate. The monoclonal antibody against DPD is added to the plate, and the free DPD in urine competes with the DPD coated on the plate for the antibody. A second antibody conjugated to alkaline phosphatase (goat anti-rabbit immunoglobulin G alkaline phosphatase) is added to the plate to bind with antibody against DPD. A substrate, p-nitrophenylphosphosphate, is added to produce a yellow color. Optical density was measured at 405 nm (30). In the conditions of this study, the lower limit of detection for the assay was 3 nmol. The intra- and interassay variations were 6 and 8%, respectively.

RESULTS

During the 105 days of the entire experimental period, body weight increased in the four groups of rats; from 312 ± 4 to 420 ± 10 g (P < 0.05) and from 304 ± 7 to 468 ± 10 g (P < 0.05) in CXE and CXR rats, respectively. Simultaneously, it increased from 301 ± 7 to 426 ± 10 g (P < 0.05) and from 301 ± 6 to 487 ± 8 g (P < 0.05) in SHE and SHR rats, respectively. However, from day 67 until day 105, running significantly decreased the rate of growth in SH and CX rats (Fig. 1).

At necropsy, the weights of the heart and lungs were significantly increased by running in both CX and SH rats, but running did not affect the size of the femur (Table 1).

Although a tendency toward higher resistance (+12%) for exercised rats was observed, the femoral failure stress was not significantly different between SHR (95 ± 4 MPa) and SHE rats (107 ± 8 MPa) and between CXR (90 ± 4 MPa) and CXE rats (101 ± 4 MPa).

At necropsy, the DBMD was not different between any of the groups. However, TBMD and MBMD were lower in CXR than in CXE (P < 0.05) or SHR (P < 0.05) or SHE (P < 0.05) rats (Fig. 2). In the same way, left femoral total Ca content was lower in CXR (235. 2 ± 3.2 mg Ca/g dry defatted bone) than in CXE (251.3 ±
2.8 mg Ca/g dry defatted bone; P < 0.05) or SHR (249 ± 0.6 mg Ca/g dry defatted bone; P < 0.05) or SHE rats (258.7 ± 3 mg Ca/g dry defatted bone; P < 0.01).

At the distal right femoral epiphysis, trabecular area and trabecular perimeter related to the digitized area (%) were lower in sedentary than in exercising rats. Furthermore, trabecular morphology (estimated as the trabecular perimeter related to trabecular surface) was modified by the experimental conditions, with the thinnest trabeculae being observed in the sedentary group and exercise increasing their thickness (Fig. 3).

In the four groups of rats, no significant change in plasma Ca concentration was observed between days 0 and 105. At the same time, plasma OC concentration significantly decreased in each group. At the time the rats were killed, it was lower in SH (24.6 ± 1.3 ng/ml) than in CX rats (29.8 ± 1 ng/ml; P < 0.05; Table 2).

Urinary DPD excretion decreased in each group of rats between days 6 and 104. It was always higher in CX than in SH rats. However, between days 33 and 104, except on day 47, in both CX and SH animals, running significantly decreased urinary DPD excretion (Fig. 4). Although mean daily urinary Ca excretion was higher in CX (2.52 ± 0.18 mg) than in SH rats (1.64 ± 0.19;
DISCUSSION

Testosterone plays an important role in the regulation of growth (26). Mechanical factors also greatly influence bone growth and remodeling (1, 11, 13, 14). Nevertheless, in our experimental conditions, the effect of exercise on growth appeared more important than that of gonadal hormones because the daily weight gain was lower in exercised than in sedentary animals during the last weeks of the experimental period (Fig. 1). Our results do not allow determination of whether body composition was different between these groups, i.e., less fat in exercised rats, so that lean body mass may not have been reduced by the exercise.

The decrease in both plasma OC concentrations (Table 2) and urinary DPD excretion (Fig. 4) observed in all the rats between days 0 and 105 indicated a decrease in bone remodeling in 5-mo-old vs. 6-wk-old rats. The mean daily weight gain measured during the first 2 wk of the experimental period (1.54 g) was about twice that measured during the last 2 wk (0.78 g). Surgical CX of male rats induces bone loss as a result of the decrease in testosterone concentration, because these bony changes are inhibited by exogenous testosterone injections (28, 38). In the same way, in the young growing male rats used in this experiment, BMD (Fig. 2) and bone Ca content (243 ± 4 mg Ca/g dry defatted bone in CX rats vs. 254 ± 3 mg Ca/g dry defatted bone in SH rats; P < 0.05) were lower in CX than in SH rats. This probably resulted from increased bone resorption after orchidectomy, as indicated by higher urinary DPD excretion in CX than in SH rats (Fig. 4). Increased osteoclastic activity, demonstrated at necropsy by higher plasma OC in CX rats (30 ± 1 ng/ml) than in SH rats (25 ± 1 ng/ml; P < 0.05), was unable to compensate for bone resorption.

DEXA has been previously employed to measure bone loss in ovariectomized rats (12, 29). A decrease in trabecular density of appendicular bones induced by orchidectomy has already been demonstrated by using histomorphometry, peripheral quantitative computed tomography, and DEXA (21, 25). Thus the most prominent effect of orchidectomy observed here on trabecular bone (Fig. 2) is in agreement with previous reports in male rats (21, 25, 28, 38).

The intensity of running was based on estimates of VO$_{2\text{max}}$ for the rats used in this experiment (18). Running can change bone morphometry in experimental animals. Cortical bone area has been shown to increase up to 23% in growing pigs after 12 mo of treadmill running (41). In our experimental conditions, treadmill running did not influence femoral morphology. Nor did it seem to affect mechanical properties of this bone, because femoral failure stress was not significantly different in exercised and sedentary rats. Forwood and Parker (8) and Wheeler et al. (39) found increases in femoral cortical area in 3-mo-old rats after a short-term high-intensity exercise program. Nevertheless, the running rats (60% VO$_{2\text{max}}$ for 1 h daily, during 3 mo) showed a higher BMD (Fig. 2) and bone Ca content than did sedentary CX animals. Such an inhibition was probably related to a decrease in trabecular bone resorption induced by exercise, because running had no significant effect on DBMD (i.e., mainly cortical) in CX rats, whereas it increased MBMD (i.e., predominantly cancellous) in these animals (Fig. 2). In fact, trabecular bone area (which was ~6% greater in CXE and SHE than in CXR and SHR rats, respectively) and volume (2% increase in CXE vs. CXR rats; 3% increase in SHE vs. SHR rats; Fig. 3) were higher in exercised than in sedentary rats. Furthermore, such an effect occurring only on trabecular bone might partly explain the lack of significant effect of treadmill running on femoral failure load observed in this experiment. This result differs from that reported by Tuukanen et al. (34), who reported that treadmill running (10 m/min, 1 h/day for 4–8 wk) did not prevent the lowered trabecular bone volume induced by orchidectomy in 3-mo-old rats but increased $^{45}$Ca incorporation into bone.

In conclusion, in young orchidectomized rats, femoral density and Ca content were higher in treadmill running (60% VO$_{2\text{max}}$ for 1 h daily for 3 mo) than in sedentary rats. Such an effect mainly resulted from an inhibition of bone resorption, as indicated by decreased urinary DPD excretion, whereas this exercise had probably no major effect on osteoblastic activity because plasma OC concentrations did not differ in exercised and sedentary rats.

The authors thank Dr. L. Hansen (Brigham Young University, Provo, Utah) for reviewing the manuscript.

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Received 22 July 1996; accepted in final form 11 March 1997.
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