# Bone metabolism and repair are normal in athymic rats

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**Several reports indicate that the thymus gland is important in the regulation of bone metabolism. Anatomic or physiologic abnormalities in the bones of athymic animals could therefore be expected. The mechanical properties, circulation, and mineralization rate of intact femora, tibiae, ossa ilia, and of an osteotomized tibia of athymic Lewis rats were compared with those of normal Lewis rats. The results were not significantly different in the two groups. The absence of the thymus thus does not seem to have any major influence on bone structure, function, or regenerative properties.**

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The immune system may be involved in the regulation of bone formation and resorption (Nisbet 1981). Several reports have suggested that the removal or the absence of the thymus gland alters the growth and metabolism of bone (Berek et al. 1968, Mandi et al. 1971, Campbell et al. 1979, Gyarmati et al. 1983). A reduction of femoral length and diaphyseal area, as well as decreased $^{35}$sulfur incorporation in the femur, has been described in neonatally thymectomized rats (Campbell et al. 1979). Moreover, a defect in bone resorption leading to osteopetrosis has been linked to a defect in the thymus gland and malfunctioning T-lymphocytes (Milhaud and Labat 1978).

If bone metabolism is influenced by the thymus gland, one would expect to find changes in animals where the thymus is congenitally absent. Mechanical properties, metabolism, and healing bones were therefore studied in athymic (rnu/rnu) rats.

## Materials and methods

A total of 32 inbred rnu/rnu rats of the Lewis strain were compared with their normal (Lew/Lew) counterparts. Male rats, 180–200 g, were used. The animals were compared at the same weight (not age) to make them more directly comparable; the athymic rats were 8 weeks old, approximately 1 week older than the normal rats.

All the animals received 1 μCi/100 g body weight $^{85}SrCl_2$ in 0.5 mL saline intraperitoneally 3 days before they were killed. The animals were anesthetized with fentanyl/fluanisone, 5 mg/kg intraperitoneally. The right carotid artery was isolated through a longitudinal incision, and a polyethylene catheter (PE-10) was introduced into the ascending aorta through the right carotid artery. The correct catheter position was confirmed at autopsy. A bolus of approximately one million $^{141}$Ce-labeled microspheres, 15 μ in diameter (NenTrac), in 1 mL saline were injected into the ascending aorta. The syringe was flushed with 1 mL saline to inject the remaining microspheres. A correct and uniform trapping of the microspheres was observed directly under the operating microscope in the iris of the animal.

In 12 animals of each strain, the tibia was osteotomized 3 weeks before death. A longitudinal incision was made over the left tibia, and an oblique osteotomy was made with a dental rotating saw under direct vision and protection of soft tissues. The tibia was stabilized with an intramedullary nail (Figure 1).

The animals were killed with an intraarterial overdose of pentobarbital. All the soft tissue and periosteum were removed from the femora, tibiae, and iliac bones. Care was taken to leave the tibial callus intact. Intramedullary nails were removed. The bones were radiographed at 45 kV, 20 mAs, and 60-cm tube-target distance using dental x-ray film (Kodak Ultra-speed D). All the samples were weighed to the nearest 0.1 mg (wet weight). The femora and the tibiae were stored at –20 °C until mechanical testing.

Before the mechanical testing, the bones were thawed, and the length and mid-diaphyseal diameter were measured with a sliding caliper. Following the

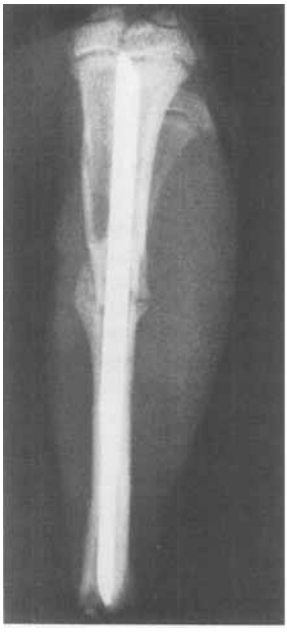

Figure 1. Left rat tibia with an osteotomy and intramedullary nail 3 weeks after the operation. The nail consists of three syringes of different sizes that have been threaded into one another to fit into the marrow canal along its entire length.

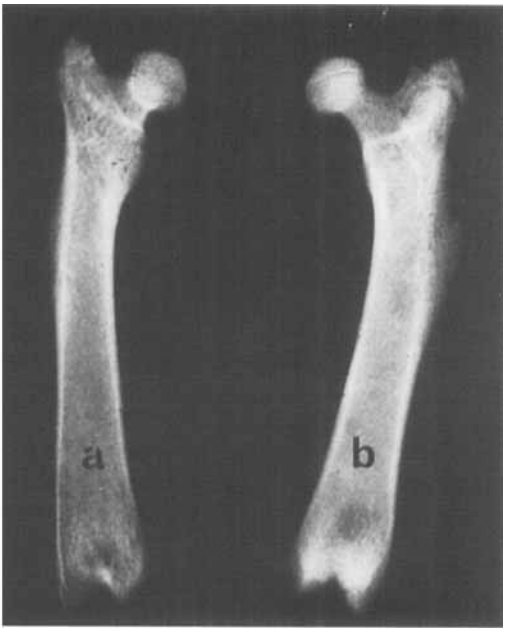


Figure 2. Femora of athymic rat (a) and normal rat (b) with the soft tissues removed; photographed together.

mechanical test, the proximal 5 mm and distal 7.5 mm of the osteotomized tibia were removed to isolate the callus segment, which was weighed to the nearest 0.1 mg. All the samples were counted for $^{85}Sr$ and $^{141}Ce$ radioactivity in a gamma scintillation counter (Hewlett-Packard). Before counting the samples, a background count and a count of a known number of microspheres were made, and the counts per minute (cpm) of one microsphere was calculated. The number of microspheres in each sample was calculated as the cpm in the bone sample/cpm per microsphere.

The bending tests were performed in the hydraulic testing machine described by Engesaeter et al. (1978). Femora, intact tibiae, and healing tibiae were tested with three-point bending in the posterior direction until failure with a deformation rate of 2.5°/s. The strength of the bones was defined as the ultimate moment, read as the y-coordinate from each load deformation curve. The corresponding x-coordinate was defined as the ultimate deformation. The stiffness was measured from the slope of the linear portion of the curves. The technique and the calculations have been described in detail by Engesaeter et al. (1978) and Ekeland et al. (1983).

The radiographic density of the femora was assessed blindly by 2 observers on a three-point scale (low, medium, high). The marrow cavity and diaphyseal width were measured with a pair of calipers on the radiographs.

The Student's *t*-test was used for the statistical evaluation, and *P*-values less than 0.05 were considered significant.

## Results

The teeth and hind-limb skeleton of the athymic rats were macroscopically indistinguishable from normal. Radiographically, the femora of athymic and normal rats were of equal density, and the diaphyseal width/marrow cavity width did not differ (Figure 2). There were no differences in the length, diaphyseal width, and wet weight of the hind-limb skeletal bones (Table 1).

The number of microspheres in the bone samples were > 1,000 for all the intact bones and > 500 in all the callus samples. The number of microspheres, used as a measure of the density of precapillary vessels, showed no difference between the two

Table 1. Length, diaphyseal width, weight, and mechanical properties of bones of athymic (rnu/rnu) and normal (Lew/Lew) rats. Mean *SD*

| | Athymic (n 20) | | Normal (n 20) | |
|---|---|---|---|---|
| Femora | | | | |
| length (mm) | 33.1 | *2.5* | 33.8 | *3.2* |
| diaphyseal width (mm) | 3.8 | *0.4* | 3.9 | *0.3* |
| weight (mg) | 667 | *93* | 664 | *86* |
| strength (Nm) | 0.474 | *0.14* | 0.472 | *0.13* |
| stiffness (Nm/degree) | 5.76 | *2.7* | 4.97 | *2.2* |
| deformation (degrees) | 4.7 | *1.8* | 5.3 | *2.1* |
| Tibiae | | | | |
| length (mm) | 22.9 | *1.3* | 23.1 | *0.7* |
| weight (mg) | 499 | *75* | 524 | *60* |
| strength (Nm) | 0.300 | *0.10* | 0.275 | *0.13* |
| stiffness (Nm/degree) | 5.5 | *3.0* | 5.3 | *3.1* |
| deformation (degree) | 4.5 | *1.8* | 4.0 | *1.1* |
| Ossa ilia | | | | |
| weight (mg) | 190 | *33* | 188 | *32* |

Table 2. Density of precapillary vessels and mineralization rate in bones of athymic and normal rats. Mean *SD*

| | Athymic (n 18) | | Normal (n 19) | |
|---|---|---|---|---|
| Femora | | | | |
| microspheres/mg | 3.8 | *1.2* | 5.0 | *2.1* |
| strontium act. /mg | 98 | *57* | 117 | *56* |
| Tibiae | | | | |
| microspheres/mg | 3.7 | *2.1* | 3.1 | *1.1* |
| strontium act. /mg | 101 | *23* | 114 | *26* |
| Ossa ilia | | | | |
| microspheres/mg | 6.5 | *3.2* | 6.7 | *3.3* |
| strontium act. /mg | 162 | *133* | 199 | *149* |

Table 3. Healing of an experimental tibial osteotomy 3 weeks postoperatively in athymic and normal rats. Mean *SD*

| | Athymic (n 12) | | Normal (n 12) | |
|---|---|---|---|---|
| Mechanical properties | | | | |
| strength (Nm) | 0.065 | *0.029* | 0.086 | *0.056* |
| stiffness (Nm/degrees) | 1.6 | *1.8* | 2.2 | *2.4* |
| deformation (degrees) | 5.4 | *4.1* | 4.7 | *2.8* |
| Callus segment | | | | |
| weight (mg) | 210 | *63* | 234 | *88* |
| microspheres/mg | 3.4 | *2.7* | 3.2 | *1.6* |
| strontium act. /mg | 154 | *64* | 162 | *49* |

groups (Table 2). Further, the activity of $^{85}Sr$, used as a measure of the mineralization rate, exhibited no difference. The mechanical properties of both femora and tibiae were likewise similar in the two groups (Table 1).

There were no differences between the two osteotomy groups: neither in mechanical parameters of healing, nor in callus weight, vessel ingrowth, or mineralization rate (Table 3). The radiographic appearance of the callus was similar in both groups.

## Discussion

The most essential functions of the skeleton are those of mechanical strength and structural support. The mechanical properties of bone are therefore important parameters. The bending test used in this study has previously given a reliable assessment of the mechanical properties of healing, as well as intact bones; and the test apparatus, the technique, and the calculations have been used in several experimental situations (Engesæter et al. 1978, Ekeland et al. 1982, 1983). The mechanical properties of the healing fractures in our study have, however, a large coefficient of variation; and differences between the two groups would have to be large to be statistically significant at this stage of healing.

An adequate blood supply is essential for all the homeostatic and regenerative properties of bone. The microsphere method is considered the most accurate technique for measuring the circulation to various organs (Heymann et al. 1977), and it is well suited for measurement of bone blood flow (Gross et al. 1981). Radioisotopes of strontium are widely used in studies of calcium metabolism of bone. Strontium uptake reflects the rate of mineralization of healing bone and mineral turnover in normal bone (Elves 1974).

There are no studies that are directly comparable to the present one; but a microscopic examination of bones in athymic mice has shown increased osteoclast activity (suggesting increased bone resorption), and has also shown a narrow epiphyseal cartilage (Gyarmati et al. 1983). Neonatally thymectomized rats have reduced $^{35}$sulfur incorporation in the femur, suggesting reduced glycosaminoglycan content (Campbell et al. 1979). The metaphyses of neonatally thymectomized mice show a sparseness of bone trabeculae (Berek et al. 1968). These studies imply an increased bone resorption in animals with thymus aplasia or dysfunction.

The data reported here cast doubt on the notion that a normal functioning thymus is necessary for the competence of osteolytic cells (Milhaud and Labat 1978). The bones of the rnu/rnu rat were morphologically and radiographically indistinguishable from those of the normal animal, although they were somewhat smaller than those of normal animals of

the same age, as were all the organs of the rnu/rnu rat (Festing 1981). This accords with the recent report of McCauley et al. (1989). They concluded, using biochemical, histomorphometric, bone ash, and in vitro studies, that physiologic bone turnover was not markedly altered in athymic mice at 6 or 12 weeks of age.

The thymus and the T-cell system have been linked to other aspects of bone development and structure (Campbell et al. 1979). Experimental models using thymectomized animals or immunosuppressive drugs have, however, given equivocal or conflicting results as regards the influence of T-cells on osteoclast activating factor (Gyarmati et al. 1983, Horowitz et al. 1984) and bone loss associated with inflammation (Guggenheim et al. 1981, Yoshie et al. 1985).

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## References

Berek L, Banos Z, Szeri I, Anderlik P, Aszodi K. Osseal changes in mice following neonatal thymectomy. *Experientia* 1968; 24(7): 721–3.

Campbell C, Lipscomb H L, Sharp J G. Influence of the thymus gland on bone growth in rats. *J Anat* 1979; 129: 871.

Ekeland A, Engesæter L B, Langeland N. Influence of age on mechanical properties of healing fractures and intact bones in rats. *Acta Orthop Scand* 1982; 53(4): 527–34.

Ekeland A, Myhre L, Underdal T. Effects of salmon calcitonin on mechanical properties of healing and intact bone and skin in rats. *Acta Orthop Scand* 1983; 54(3): 462–9.

Elves M W. An evaluation of the use of strontium$^{85}$ for the assessment of experimental bone grafts. *Acta Orthop Scand* 1974; 45(5): 641–51.

Engesæter L B, Ekeland A, Langeland N. Methods for testing the mechanical properties of the rat femur. *Acta Orthop Scand* 1978; 49(6): 512–8.

Festing M W F. The Rowett athymic nude rat. In: *Thymusplastic nude mice and rats in clinical oncology* (Ed. Bastert G B A). Gustav Fisher Verlag, Stuttgart 1981; 1: 15–23.

Gross P M, Marcus M L, Heistad D D. Measurement of blood flow to bone and marrow in experimental animals by means of the microsphere technique. *J Bone Joint Surg* (Am) 1981; 63(6): 1028–31.

Guggenheim B, Gaegauf Zollinger R, Hefti A, Burckhardt J J. The effect of cyclosporin A on periodontal disease in rats monoassociated with Actinomyces viscosus Ny 1. *J Periodont Res* 1981; 16(1): 26–38.

Gyarmati J Jr, Mandi B, Fachet J, Varga S, Sikula J. Alterations of the connective tissue in nude mice. *Thymus* 1983; 5(5–6): 383–92.

Heymann M A, Payne B D, Hoffman J I, Rudolph A M. Blood flow measurements with radionuclide labeled particles. *Prog Cardiovasc Dis* 1977; 20(1): 55–79.

Horowitz M, Vignery A, Gershon R K, Baron R. Thymus derived lymphocytes and their interactions with macrophages are required for the production of osteoclast activating factor in the mouse. *Proc Natl Acad Sci U.S.A.* 1984; 81(7): 2181–5.

Mandi B, Hadhazy C, Mandi A, Glant T. Effect of postnatal thymectomy on enchondral ossification. *Acta Morphol Acad Sci Hung* 1971; 19(3): 259–68.

McCauley L K, Rosol T J, Capen C C, Horton J E. A comparison of bone turnover in athymic(nude) and euthymic mice: biochemical, histomorphometric, bone ash and in vitro studies. *Bone* 1989; 10(1): 29–34.

Milhaud G, Labat M L. Thymus and osteopetrosis. *Clin Orthop* 1978; 135: 260–71.

Nisbet N W. Bone absorption and the immune system. *Scand J Immunol* 1981; 4(6): 599–605.

Yoshie H, Taubman M A, Ebersole J L, Smith D J, Olson C L. Periodontal bone loss and immune characteristics of congenitally athymic and thymus cell reconstituted athymic rats. *Infect Immun* 1985; 50(2): 403–8.