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# Femur Mass: Modulation by Marrow Cells from Young and Old Donors (40829)[1]

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A universal loss of bone mass normally begins in the third or fourth decade of life, affecting cancellous trabecular bone to a greater degree than cortical bone (1, 2). When severe, this bone loss causes increased susceptibility to fractures of vertebrae and long bones. It is estimated that a minimum of 10% of the population over the age of 50 have acute or chronic disabilities as a result of these boney changes.

Although the consequences of this age-related relentless decrease in bone mass are well known, the etiology and pathogenesis of the disorder are unclear (3–5). Dietary indiscretion, inactivity, decreased muscle mass, hormonal imbalance, renal dysfunction, vitamin deficiencies, and defects in intestinal absorption have been implicated as causative factors. However, the results of all therapeutic measures based on these premises have resulted in limited, if any, overall symptomatic improvement in skeletal pain or reduction in the incidence of fractures in the population at risk.

Recently, several lines of investigation have suggested the possibility that age-related osteopenia may be the result of an intrinsic disorder or imbalance of marrow-derived cells which regulate bone formation and remodeling rather than disorders of mineral metabolism or of osteoblasts and osteoclasts per se. First, it has been shown *in vitro* that bone marrow and peripheral blood lymphoid cells from humans and mice can secrete a soluble factor which stimulates bone resorption (osteoclast-activating factor, OAF, 6, 7). Bone resorption also can be produced *in vitro* by direct contact with human monocytes (8, 9) and by synthetic or macrophage-secreted prostaglandins (10, 11).

Second, it has been demonstrated that the capacity to resorb the excessive bone and calcified cartilage found in mice (12–14) and rats (15, 16) with inherited osteopetrosis can be restored by transplanting marrow or spleen cells from normal litter mates to the affected animals. Further, osteopetrosis can be induced in normal young mice by transferring marrow cells from affected sibs (17). Based on this evidence, studies were initiated to determine if age-related bone loss is due to changes in the cells of the marrow compartment.

*Materials and methods.* $B6D2F_1$ and $B6AF_1$ female mice were obtained at 6 to 8 weeks of age from The Jackson Laboratory, Bar Harbor, Maine, and they were maintained on Purina Laboratory Chow and water *ad lib*. In normative studies, mice were killed at various ages; their femurs were removed intact, autoclaved, defleshed, defatted, dried, and femur weight, ash weight, and total calcium were determined (18). It was found that femur and femur ash weights (Fig. 1) of $B6D2F_1$ female mice peak between 8.5 and 13 months and begin to decline at about 20 months. At 25 months of age, femur weight has decreased approximately 8% from peak values and ash weight and total calcium have declined 11 to 12%. There does not appear to be a significant change with age in the ratio of calcium to dry femur weight (Fig. 1); this is consistent with the type of bone loss seen in human osteoporosis (1). Although the data are incomplete, femur and femur ash weights (Fig. 2) of $B6AF_1$ female mice peak between 10 and 19 months and by 35 months of age ash weight has decreased by 16%.

To investigate the effects of young and old marrow cells on bone mass, 3- to 19-month-old mice were given 900r X-radiation (300 kvp, 20 mA, HVL 0.1 mm Cu), and within 2 hr they were injected intravenously with 5 to $10 \times 10^6$ syngeneic bone marrow cells taken from healthy donors 2 to 36 months of age (controls were given marrow cells from mice the same age

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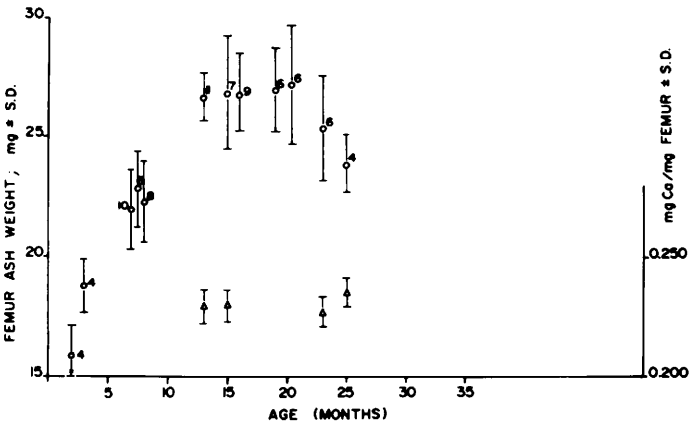


FIG. 1. Femur ash weight (0,*n*) and milligrams Ca/milligram dry femur (Δ) of B6D2F$_1$ mice at various ages.

as the host). They were killed 4 to 6 months later, and femur mass was determined.

*Results and discussion.* When 3-month-old mice were given marrow cells from 23 or 36 month old donors (Table 1, Expt. I–III), femur weights were reduced 8.8, 9.7, and 7.5% and ash 5, 15.6, and 12.8% below values obtained in mice given marrow from 3-month-old mice (in Expt. III total calcium was reduced 11.6%). In Experiment IV, marrow from an 18-month-old donor appeared to produce a small decrease in the femur mass of 8-month recipients (femur weight, −6.6%; ash, −4.8%; calcium, −12%). Clearly, old marrow cells retard bone growth in young mice. Unfortunately, it cannot be stated that bone loss has occurred in these experiments.

When 12-month-old mice were given marrow cells from 23- (Expt. V) or 36-month-old (Expt. VI) mice, femur weights were reduced 4.8 and 14%, ash weights 10.6 and 23.2%, and total calcium 9.4 and 18.2% when compared to hosts given marrow from mice 1 year old. This clearly shows that bone mass can be reduced significantly by marrow from old mice. One-year-old hosts given marrow cells from 3-month-old donors had slightly greater femur, ash, and total calcium weights than did those given

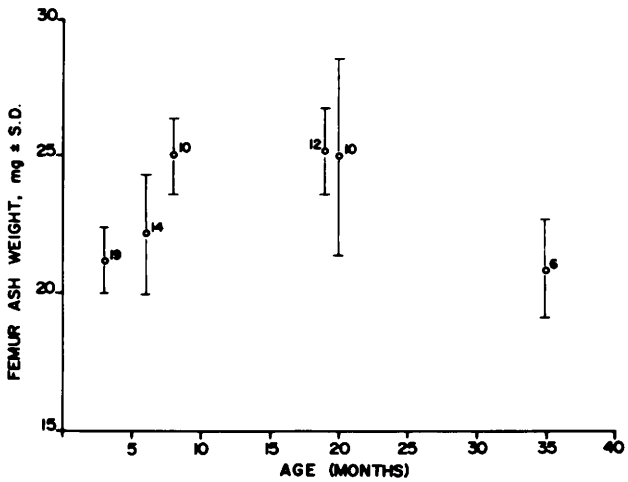


FIG. 2. Femur ash weight (0,*n*) of B6AF$_1$ female mice.

TABLE I. MODULATION OF BONE MASS BY MARROW CELLS

| Expt strain | Age of host (months)[a] | Age of donor (months)[a] | No. of femurs | Weight (mg ± SD) Dry femur | Ash | Total calcium |
|---|---|---|---|---|---|---|
| I (4),[b] $B6D2F_1$ | 3 | 3 | 14 | 39.8 ± 2.0[f] | 23.9 ± 1.3[d] | 9.8 ± 0.6[d] |
| | 3 | 23 | 8 | 36.3 ± 2.3[f] | 22.7 ± 1.6[d] | 9.2 ± 0.6[d] |
| II (4), $B6AF_1$ | 3 | 3 | 16 | 40.3 ± 2.6[f] | 26.3 ± 1.7[f] | 9.3 ± 0.5[f] |
| | 3 | 36 | 20 | 36.4 ± 3.3[f] | 22.2 ± 2.1[f] | 8.7 ± 0.4[f] |
| III (4), $B6AF_1$ | 3 | 3 | 9 | 38.7 ± 2.1[f] | 24.3 ± 1.5[f] | 7.8 ± 0.2[f] |
| | 3 | 36 | 9 | 35.8 ± 2.0[f] | 21.2 ± 0.4[f] | 6.9 ± 0.3[f] |
| IV (6), $B6D2F_1$ | 8 | 8 | 10 | 39.8 ± 2.9[d] | 23.3 ± 2.0[c] | 9.3 ± 0.8[c] |
| | 8 | 18 | 11 | 37.2 ± 3.5[d] | 22.2 ± 2.5[c] | 8.2 ± 0.9[c] |
| V (4), $B6D2F_1$ | 12 | 3 | 17 | 43.5 ± 1.8[f,c] | 27.4 ± 1.7[f,c] | 10.7 ± 0.4[f,d] |
| | 12 | 12 | 15 | 41.9 ± 3.6[c,c] | 26.6 ± 2.4[f,c] | 10.2 ± 0.8[d,d] |
| | 12 | 23 | 11 | 39.9 ± 2.3[f,c] | 23.8 ± 1.4[f,f] | 9.7 ± 0.6[f,d] |
| VI (6), $B6AF_1$ | 12 | 12 | 10 | 38.5 ± 3.4[f] | 22.0 ± 2.1[f] | 8.8 ± 0.8[f] |
| | 12 | 36 | 6 | 33.1 ± 1.1[f] | 16.9 ± 0.2[f] | 7.2 ± 0.7[f] |
| VII (6), $B6AF_1$ | 14 | 14 | 14 | 39.1 ± 2.4[c] | 24.2 ± 1.6[c] | 9.3 ± 0.5[c] |
| | 14 | 2 | 17 | 39.9 ± 4.2[c] | 24.3 ± 2.8[c] | 9.3 ± 0.4[c] |
| VIII (6), $B6D2F_1$ | 18 | 18 | 9 | 40.6 ± 2.8[c] | 25.2 ± 1.6[c] | 8.9 ± 0.7[c] |
| | 18 | 8 | 9 | 41.1 ± 1.8[c] | 26.2 ± 1.7[c] | 9.1 ± 0.5[c] |
| IX (6), $B6D2F_1$ | 19 | 19 | 12 | 39.7 ± 2.2[c] | 24.7 ± 1.4[d] | 8.1 ± 0.6[d] |
| | 19 | 9 | 10 | 41.6 ± 3.0[c] | 26.9 ± 1.3[d] | 8.9 ± 0.4[d] |

*Note.* $B6D2F_1$ and $B6AF_1$ female mice were given a lethal dose of X-radiation and protected with marrow cells from syngeneic mice of various ages. Four to six months later femur mass was determined.

[a] Age at time of irradiation and marrow transfer.

[b] Months from irradiation to sacrifice.

[c] $P > 0.05$, Student's t test.

[d] $P < 0.05$.

[e] $P < 0.02$.

[f] $P < 0.01$.

1-year-old marrow cells (Expt. V), but the differences were marginally significant. Similarly, because bone mass is normally stable between 12 and 20 months of age, no significant differences were expected when 14-month-old mice were given marrow from 2- or 14-month-old donors, and none were found (Expt. VII).

It was anticipated, however, that transfer of marrow cells from 8- to 9-month-old donors to 18- to 19-month-old hosts would prevent and/or reverse the bone loss that would normally occur over the 6-month experimental period. In one study this was found to be true (Expt. IX) but in the other (Expt. VIII), although bone mass was slightly increased in the group receiving young cells, the increases were not significant.

Taken together these studies provide a new paradigm for the study of human osteoporosis, and the data show that bone growth can be retarded and bone loss produced in young and mature animals by the transplantation of marrow cells from old donors. The observations also suggest that the bone loss seen in aging animals can be prevented or reversed by young marrow cells. The marrow cells and/or factors which mediated the boney changes seen in these experiments remain to be defined as does the relationship between this model and the osteopenia seen in humans.

*Summary.* Mice were given a lethal dose of X-radiation and protected with marrow cells from young or old donors. Four to six months later femur mass was determined. Femur mineral mass was decreased when marrow cells from old mice were transplanted into young or mature recipients and increased when young marrow cells were given to aging mice.

1. Avioli, L. V., Advan. Internal Med. **21**, 391 (1976).
2. Wheeler, M., Med. Clin. N. Amer. **60**, 1213 (1976).
3. Berlyne, G. M., Ben-Ari, J., Kushelevsky, A., *et al.*, Quart. J. Med. **44**, 505 (1975).
4. Avioli, L. V., *in* "Nutritional Disorders of American Women" (M. Winick, ed.). Wiley, New York (1977).
5. Editorial, Lancet **1**, 181 (1976).
6. Mundy, G. R., Raisz, L. G., Cooper, E., *et al.*, New Engl. J. Med. **291**, 1041 (1974).
7. Raisz, L. G., Luben, R. A., Mundy, G. R., *et al.*, J. Clin. Invest. **56**, 408 (1975).
8. Mundy, G. R., Altman, A. J., Gondek, M. D., and Bandelin, J. G., Science **196**, 1109 (1977).
9. Kahn, A. J., Stewart, C. C., and Teitelbaum, S. L., Science **199**, 988 (1978).
10. Tashijian, A. H., Tice, J. F., and Sides, K., Nature (London) **266**, 645 (1977).
11. Santoro, M. G., Jaffee, B. M., and Simmons, D. J., Proc. Soc. Exp. Biol. Med. **156**, 373 (1977).
12. Walker, D. G., Endocrinology **91**, 916 (1972).
13. Walker, D. G., Science **190**, 784 (1975).
14. Loutit, J. F., and Sansom, J. M., Calc. Tissue Res. **20**, 251 (1976).
15. Marks, S. C., Amer. J. Anat. **146**, 331 (1976).
16. Toyama, K., Moutier, H., and Lamendin, H. C., C. R. Acad. Sci. **276**, 115 (1974).
17. Walker, D. G., Science **190**, 785 (1975).
18. Krishnarao, G. V. G., and Draper, H. H., J. Nutr. **102**, 1143 (1972).

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