Progressive Cancellous Bone Loss in Rats after Adrenalectomy and Oophorectomy

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Summary. Female Sprague-Dawley rats (n = 72), 6 months old, underwent either sham operation, oophorectomy, adrenalectomy, or combined oophorectomy and adrenalectomy (O&A). They were all maintained on normal saline *ad libitum* and 20 g/day 1.1% calcium chow. Nine weeks after operation, the trabecular bone volume of the distal femoral shaft was significantly lower ($P < 0.001$) in the adrenalectomized (11.1%), oophorectomized (7.0%), and O&A (8.3%) animals than in sham-operated animals (19.8%). Eighteen weeks after operation, the trabecular bone volume in O&A animals had fallen to a mean of 3.8% (sham 17.0%), and the length of the femur had increased to 38.8 mm after O&A (sham 36.8 mm, $P < 0.01$). O&A animals treated with 0.35 mg/kg/week nandrolone decanoate from 9 weeks postoperatively onward, had twice the femoral trabecular bone volume of untreated animals at 18 weeks ($P < 0.05$). By contrast, no significant differences were found in vertebral body trabecular bone between any groups, including groups receiving treatment with androgens. We have found that, by 9 weeks after operation, adrenalectomy alone causes significant loss of metaphyseal trabecular bone, similar to the progressive loss seen after oophorectomy.

Key words: Osteoporosis — Androgens — Adrenopause — Menopause.

Loss of ovarian function at the menopause is generally accepted as causing osteopenia, and bone loss after oophorectomy has been amply demonstrated in the rat [1, 2]. However, oophorectomy is just one of several ways of inducing bone loss in rats, and the menopause is unlikely to be the only factor leading to osteoporosis in elderly Caucasian women. The crucial factors are difficult to disentangle as any of a number of small but sustained factors might produce insidious net bone loss. After oophorectomy, the output of cortisol (or corticosterone in the rat) and of estrogenic and anabolic metabolites of dehydroepiandrosterone from the adrenal cortex might be of consequence [3–5]; indeed, the rat adrenal cortex shrinks after oophorectomy perhaps through overuse [6]. Osteoporosis might follow from a decrease in production of androgenic adrenal metabolites after the menopause [7], described as the adrenopause.

The possibility that deficiency of some adrenal androgens leads to osteopenia, which was originally suggested by Albright et al. [8], might be inferred from the temporal association between the age-related decline in dehydroepiandrosterone sulfate (DHEAS) and the decline in bone density which also accompanies aging [9, 10], but experimental evidence of a direct causative link has yet to be found. An animal model demonstrating an effect of adrenalectomy on bone would be useful.

The vertebral bone in the tail of the rat bears a morphological resemblance to that of human iliac crest [11], and vertebral osteopenia after oophorectomy has been described in the rat [12]. This encouraged us to use rats, particularly as they usually survive adrenalectomy without mineralocorticoid if saline is substituted for drinking water. A disadvantage of rats is that they continue to grow, perhaps more vigourously after oophorectomy [13], so that effects on bone modeling are likely to be confounded with those on remodeling.

Osteopenia after oophorectomy has been shown

to occur in long bones, particularly metaphyseal trabecular bone, in the rat [13] as well as in other species including the Beagle dog [14]; this has become a favored site for demonstrating experimental bone loss. We therefore planned to establish whether adrenalectomy causes osteopenia of the distal femoral shaft and the caudal vertebral body in rats. The experimental design used is similar to that of Schot and Schuurs [15] in which nandrolone decanoate was shown to increase bone mass in oophorectomized rats.

Methods

Adult female Sprague Dawley rats (n = 108), 27 weeks old and with mean body weight of 302 g, were allocated to nine groups and treated as follows: group 1, sham-operated rats, killed after 9 weeks; group 2, sham-operated, killed after 18 weeks; group 3, oophorectomized rats, killed after 9 weeks; group 4, adrenalectomized rats, killed after 9 weeks; group 5, combined oophorectomized and adrenalectomized (O&A) rats, killed after 9 weeks; group 6, O&A rats, given no drug, killed after 18 weeks; group 7, O&A rats, given nandrolone decanoate 0.35 mg/kg i.m. weekly after 9 weeks, killed after 18 weeks; group 8, O&A rats, given DHEAS 2.33 mg/kg i.p. weekly (by twice weekly injection) after 9 weeks, killed after 18 weeks; group 9, O&A rats, given DHEAS 23.3 mg/kg i.p. weekly (by twice weekly injection) after 9 weeks, killed after 18 weeks.

In order to prevent the weight gain that follows oophorectomy, the rats were pair fed with 20 g commercial rat chow/animal/day. The chow contained 11 mg/g calcium and 2 IU/g of vitamin D_3, providing an average dietary intake of calcium of 200 mg/day. All drank 0.9% saline ad libitum postoperatively; this was a prerequisite in the adrenalectomized rats, and was extended to the other groups, producing hypernaturia and an associated hypercalciuria in all experimental groups, as will be described elsewhere.

In addition to macroscopic examination of ovaries and adrenals at death, serum corticosterone was measured by radioimmunoassay (cv 10% @ 200 nmol/liter) to assess the adequacy of adrenalectomy. In serum collected at death, the sham and oophorectomized animals all had serum corticosterone values >100 nmol/liter, and adrenalectomized or O&A animals typically had values of <20 nmol/liter; 50 nmol/liter corticosterone was selected as the criterion of effective adrenalectomy. DHEAS values were measured by radioimmunoassay (cv 12% @ 10 nmol/liter) in serum collected at death.

Both hind legs were excised, and the femora dissected for measurement of length. These and the first caudal vertebral body were fixed in 10% neutral buffered formalin for 24 hours. A 100 μm transverse section of the midfemoral diaphysis was cut with a Buehler Isomet slow speed saw with a diamond wafering blade (11-1180 Buehler Ltd. IL), for assessment of cortical area and width by planimetry, using a Hewlett-Packard 9874A digitizer and 9000 series 217 computer. The distal third of the femur was bisected longitudinally with the slow speed saw, and half was decalcified in pH 7.2 phosphate buffered 2.5% EDTA solution and processed into paraffin wax. Three serial sections were taken, spaced 200 μm apart, and stained with van Gieson's solution for assessment of trabecular bone volume as a percentage of can-

cellous tissue volume (BV/TV, old nomenclature TBV%), using an ocular mounted Weibel II grid for one observer (RJM) to make point count estimates in the area of metaphyseal bone extending from the distal third of the shaft down to the growth plate, previously described by Faugere et al. [13]. No measurements were made of the cartilagenous growth plate. Measurements of BV/TV were not corrected for differences in femoral length or other cortical bone dimensions. The caudal vertebra was bisected longitudinally with the slow speed saw, and a 200 μm slice was cut and processed into glycol methyl methacrylate resin; BV/TV was estimated on von Kossa stained sections in the area described by Baron et al. [11] using a Quantimet 520 image analyzer, again by one observer (IHP). The observers were presented with all slides without knowledge of experimental group.

Statistical analyses of differences among groups were performed using analysis of variance or t-test (Macintosh SYSTAT 3.2), with the mean and one SD or one SEM presented here, except for serum DHEAS values which are presented as the median and range. The length and BV/TV results given for DHEAS-treated rats are after amalgamation of data for the two dosages used in groups 8 and 9 (which showed no significant differences). Tukey's test was used in comparing mean values for significance of differences after analysis of variance.

Results

Of 108 animals entering the experiment, 36 were withdrawn: 28 had corticosterone values of >50 nmol/liter after "adrenalectomy" and were therefore regarded as having had an incomplete operation; 8 animals died postoperatively (all after adrenalectomy). Completeness of oophorectomy and/or adrenalectomy was confirmed at necropsy in the remaining 72 animals.

The mean body weight at death was 317.2 g (SD 30.5). Analysis of variance showed that no significant weight gain had occurred on the restricted diet during the experiment, and that there were no significant differences in body weight between the experimental groups at 9 weeks or at 18 weeks after operation.

With regard to cortical bone growth, planimetry of the femoral midshaft cortical bone area and width showed no significant differences among any of the experimental groups. However, there was a tendency to longer femora by 9 weeks after adrenalectomy or O&A: this reached statistical significance by 18 weeks after O&A, as shown in Table 1. Comparison of femur length of the O&A and sham animals at 9 and 18 weeks demonstrated that femora were longer by 1.1 mm on average after O&A: two way analysis of variance showed a trend ($P = 0.07$) to increasing length between 9 and 18 weeks, and a $P < 0.01$ difference in length between sham and O&A operations. There were no significant differences among drug treatments after O&A at 18 weeks.

Table 1. Mean (and SEM) femur lengths in millimeters

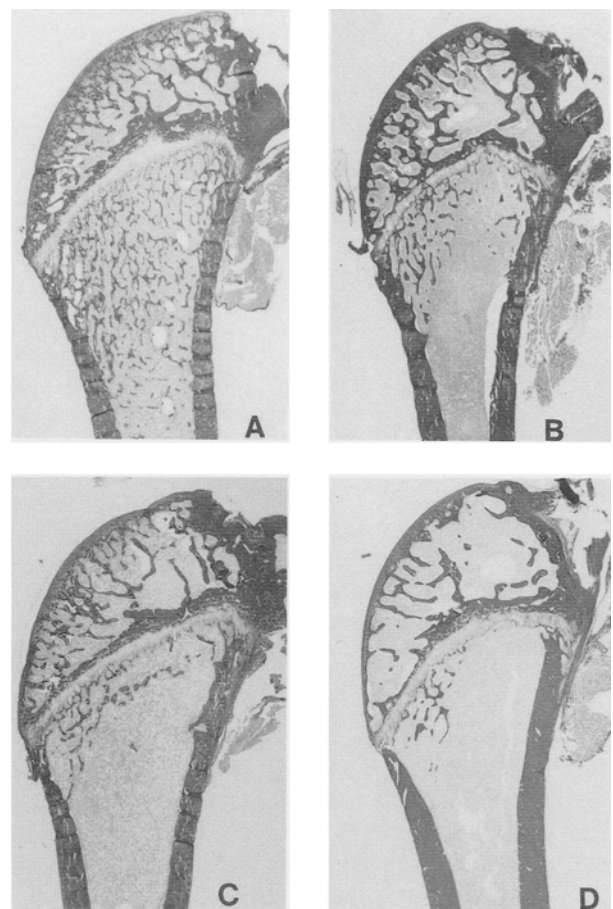|	Sham	Oophorectomy	Adrenalectomy	O&A	O&A and nandrolone	O&A and DHEAS
Weeks						
9	36.81[a]	36.89[a]	37.38	37.47	—	—
	(0.35)	(0.33)	(0.25)	(0.31)		
18	36.76[a]	—	—	38.36	38.26	37.83
	(0.30)			(0.33)	(0.26)	(0.25)

[a] Femora were shorter ($P < 0.05$) after sham operation and after oophorectomy than at 18 weeks after O&A (with or without androgen treatment)

Trabecular bone volume (BV/TV) was estimated at two sites, the distal femoral shaft and a caudal vertebral body, showing different results: As shown in Figure 1, considerable bone loss had occurred in the femur by 9 weeks after adrenalectomy, with absence of trabeculae from the midshaft down virtually to the growth plate. There was also significant bone loss in the oophorectomized and O&A animals by 9 weeks but no significant differences were found in BV/TV among the adrenalectomized, oophorectomized, and O&A groups (Table 2). Some further loss of trabecular bone was found between 9 and 18 weeks ($P < 0.05$), when sham BV/TV reached 17.0% and O&A BV/TV 3.8%.

Treatment of O&A animals between 9 and 18 weeks with nandrolone decanoate yielded a significantly ($P < 0.05$) higher BV/TV of 9.0%, as compared with untreated controls (3.8%) at 18 weeks, as shown in Table 3. This value, 9.0%, was not significantly different from the BV/TV of 8.3% found in O&A animals at 9 weeks, before nandrolone decanoate was begun.

Sham-operated animals had median serum DHEAS at death of 7 nmol/liter (range 0–59). All untreated O&A rats had undetectable levels of DHEAS. With DHEAS administration from 9 to 18 weeks, the DHEAS values ranged from 0 to 12 for animals on 2.33 mg/kg/week, and from 0 to 6 for animals on 23.3 mg/kg/week. Four of the 6 animals on the higher dose had undetectable DHEAS values. The mean BV/TV for all DHEAS-treated rats was 4.0% at 18 weeks, and no significant effects on BV/TV were found.

At the vertebral body site, in contrast, the mean BV/TV was 22.70% (SD 6.94) in the whole set of rats, and there were no significant differences among any of the experimental groups at 9 or 18 weeks, though there was a trend (*t*-test, $P = 0.07$) towards a higher vertebral BV/TV at 18 weeks after O&A (mean 23.38%) than after sham operation (mean 20.69). Bone surface and trabecular thick-

Fig. 1. Longitudinal sections of the distal femoral shaft. (**A**) Sham operation, (**B**) adrenalectomy, (**C**) oophorectomy 9 weeks postoperatively, (**D**) 18 weeks after combined oophorectomy and adrenalectomy (O&A). All sections, stained by Van Gieson's method, show cortical and cancellous bone darkly stained, with marrow showing lighter. The pale staining cartilaginous growth plate can be seen separating the epiphysis from the metaphyseal cancellous bone which has been largely lost in B, C, and D. The pattern of bone loss was similar in the other groups: DHEAS-treated O&A at 18 weeks resembled D, nandrolone-treated O&A at 18 weeks and untreated O&A at 9 weeks resembled C in the extent and distribution of metaphyseal bone loss.

Table 2. Femoral metaphyseal trabecular bone volume 9 weeks after operation

	Sham	Oophorectomy	Adrenalectomy	O&A
Mean	19.78[a]	6.79	11.05	8.27
(SEM)	(1.96)	(0.61)	(1.62)	(1.58)
Number	12	12	8	6

[a] Higher bone volume was found in the sham than in the other groups ($P < 0.001$)

Table 3. Femoral metaphyseal trabecular bone volume 18 weeks after O&A

	Drug given		
	None	Nandrolone	DHEAS
Mean	3.84	9.00[a]	4.59
(SEM)	(0.31)	(1.99)	(0.74)
Number	5	6	13

[a] Higher bone volume on nandrolone decanoate ($P < 0.05$)

ness measurements were found to show no significant differences among any experimental groups.

Discussion

We have shown that adrenalectomy alone causes significant femoral trabecular bone loss by 9 weeks after operation, under the conditions of this experiment on 6-month-old rats. The wholesale disappearance of trabeculae in the femoral shaft was systematic and progressive. It showed a consistent pattern of graded proximal to distal bone loss; and a transition zone between bone-free marrow in the central shaft and cancellous bone shifted closer to the epiphyseal growth plate. This pattern of loss after adrenalectomy was indistinguishable from that seen 9 weeks after oophorectomy, or after combined O&A, and is similar to that shown elsewhere after oophorectomy [13]. However, we have not been able to confirm the previously described vertebral body osteopenia after oophorectomy [12].

As we shall be reporting elsewhere, the saline regime (necessitated by adrenalectomy in some groups) was associated with increased urinary loss of sodium and calcium which might have contributed to the magnitude of femoral bone loss. In fact, we found a femoral BV/TV of 17.0% at 45 weeks for sham-operated animals, and Malluche's untreated sham-operated rats had a BV/TV of 10% at 52 weeks [13]. This suggests that our rats were not substantially calcium deprived despite hypercalci-

uria. The femoral BV/TV of 8% after oophorectomy we found in our rats was not lower than that found elsewhere [12, 13]. It seems unlikely that the saline regime affected our results.

We have demonstrated significantly longer femora 18 weeks after O&A, indicating cortical bone growth (not detected in sham-operated animals, and despite static body weight), and the metaphyseal osteopenia could represent abnormal modeling rather than remodeling, and hence be regarded as a disorder of growth rather than osteoporosis. However, disappearance of cancellous bone along the shaft of long bones is, as a rule, more in keeping with senescence than with growth; at 1 year old, rats have metaphyseal BV/TV around 10.0% [13], considerably lower than the youthful BV/TV of around 27% at 3 months [12]. If loss of trabeculae was related to growth, one might expect the loss to occur near the growth plate, where endochondral ossification and vigorous remodeling take place, at first sparing the previously formed trabeculae towards the midshaft.

We wish to emphasize that the femoral bone loss was not randomly distributed and was not due to a uniform change leading to, say, reduced trabecular thickness. We hypothesize that the bone loss, triggered by any one of a number of factors, is selective with regard to the site of trabeculae removed, and is progressive, leading eventually to endosteal cancellization of the cortex with advancing age, as in human osteoporosis.

After osteopenia was established by 9 weeks, treatment of O&A animals between 9 and 18 weeks with nandrolone decanoate prevented further bone loss. Hahn [16] has recently noted that present evidence indicates that the effects of androgens on bone cell metabolism are indirect; our experiment does not establish the mechanism by which bone loss was prevented. We have found no effect on bone volume with DHEAS, and we believe that it was inefficacious because even the higher dose given failed to sustain physiological serum DHEAS levels. In our own experience, such a dose given to human volunteers yields a substantial rise. The metabolic clearance rate of DHEAS by the rat has been shown to be significantly greater than for humans [17]. This could explain the DHEAS values we have found, and reflects yet another way in which rats and humans differ. DHEAS should be given more frequently to ensure adequate therapeutic concentrations in future experiments with rats.

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