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Bone changes after castration in rats.

A model for osteoporosis

Bone changes 6–12 weeks after castration have been studied in 25 female and 27 male middle-aged rats. Castrated female rats gained more weight than their controls, but had decreased bone density and calcium and hydroxyproline content per cm^3 bone volume of tibia. Castrated male rats did not differ from controls regarding body weight and the bone parameters. No influence of castration on the mechanical strength of the femora could be detected in either sex. At 2 weeks after castration, the circulating levels of immunoreactive calcitonin (iCT) were decreased in female rats compared to controls. In contrast, iCT was increased both in castrated male and female rats 10 weeks later. We conclude that castration of 6-month-old female rats causes osteoporosis, and therefore represents a promising experimental model for studying postmenopausal bone loss.

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In animals, osteoporosis can be induced via different procedures such as calcium-deficient diets (Hefti et al. 1982), injection of heparin (Ambrus et al. 1978), cortisone (Adams & Jowsey 1967) and ammonium chloride (Barzel 1975) administration, and immobilization (Izawa et al. 1981). Although metabolic bone changes have been documented after castration in rats (Langeland 1975a), only a few studies (Saville 1969, Burkart & Beresford 1978, Wink & Felts 1980, Kalu 1983) have been found where osteoporosis was developed in castrated animals.

We report the bone changes induced by castration of middle-aged rats. Because calcitonin (CT) is a bone-preserving hormone (Copp 1979, Talmage & Cooper 1979), which may play a role in the development of osteoporosis (Johnston & Epstein 1982), the plasma concentrations of this hormone were followed after castration.

Materials and methods

Experimental animals. Twenty-five female and 27 male Wistar rats were used. At the beginning of the study the female rats were 6 months old and had a mean body weight of 300 g. The male rats were 9 months old and had a mean body weight of 500 g. Three to four rats were kept in each cage. They were given standard animal pellets containing 0.9 per cent calcium and 0.7 per cent phosphorus, and water *ad libitum*.

The animals were divided into castrated and control groups. The castration was performed transabdominally under ether anaesthesia. The rats in the control groups were sham-operated by opening and closing the abdominal wall.

One week before and 2 and 10 weeks after castration, blood was collected from the tail under ether anaesthesia for determination of serum immunoreactive CT (iCT). At 12 weeks after castration, blood was sampled by aortic puncture. The samples were collected at 9 a.m. They were centrifuged immediately, and the plasma was stored at −20°C until assayed for iCT.

At 6, 8, 10 and 12 weeks after castration, groups of animals were killed by aortic exanguination in ether anaesthesia. The right tibia and femur were dissected free. The tibiae were used to determine the dry weight, the bone volume, the bone density, and the calcium and hydroxyproline concentrations of the bone. The femora were stored at −20°C until subjected to mechanical tests.

Biochemical and mechanical analyses. The tibiae were weighed in air and in distilled water at 4°C. Thereafter, the bone volumes were calculated by Archimedes' principle (Arnold 1969). After removal of the proximal and distal epiphyses, the tibial specimens were dehydrated in acetone and dried in air until constant weight (dry weight) was reached, and then hydrolyzed in 6M HCl for 18 h (Firschein 1969). The calcium concentration in the bone hydrolysates was measured with an Abbot Bichromatic Analyzer

(Gürkan et al. 1983). The concentration of hydroxyproline was determined colorimetrically in the hydrolysates (Firschein 1969).

CT was assayed radioimmunologically in plasma using rabbit antisera directed against both N-terminal and C-terminal aminoacid sequences of the human hormone (Gautvik et al. 1976, Myhre & Gautvik 1979). As rat and human CT have similar structure (Raulais et al. 1976), this assay has proved to be a reliable measure for rat CT (Normann et al. 1977).

After thawing, the femora were tested in torsion to failure (Engesæter et al. 1978). The strength of the bones defined as the ultimate torsional moment, was obtained from the load deformation curves (Ekeland et al. 1981).

Statistics. Mean and standard deviation were used to express the average and the dispersion of the measured values. Differences were evaluated by the two-way Anova and Student-Newman-Keuls tests (bone data) (Sokal & Rolf 1969), and by Student's *t* test for two samples and paired differences (body weight and CT data) (Guttman & Wilks 1965). The differences were considered significant when $p \leq 0.05$.

Results

Body weight. Castration did not alter the body weight of male rats compared to controls. Castrated female rats, however, increased their body weight (Table 1).

Biochemics. There was no change in the bone density of male rats after castration, whereas female rats had a 5 per cent reduction ($P < 0.005$) 12 weeks after the operation (Table 2).

The calcium and hydroxyproline concentrations showed similar changes in castrated female rats with reductions of about 6 per cent compared to controls 12 weeks after castration (Table 2). The corresponding values were simi-

Table 1. Body weight (g) of castrated and control rats (Mean ± SD). n = Number of animals in each group. Significant differences between the groups are indicated.

Time	n	Male		Female		
		Castrated	Controls	Castrated		Controls
At castration	12–13	513±15	558±49	297±22		294±20
12 weeks after castration	4–6	564±31	560±51	335±23	$P < 0.05$	302±30

Table 2. Bone density, calcium concentration and hydroxyproline concentration of right tibia from female and male rats after castration. Plasma calcitonin before and after castration is also given (Mean ± SD). The rats killed at 6 and 8 weeks after castration are grouped together. n = Number of animals in each group. Significant differences between the groups are indicated.

Weeks after castration	n	Bone density (10^2g/cm^3)		Calcium concentration (10 mmol/cm^3)		Hydroxyproline concentration (10^2 mmol/cm^3)		Plasma calcitonin (10^2 ng iCT/ml)	
		Castrated	Controls	Castrated	Controls	Castrated	Controls	Castrated	Controls
Female									
−1	12							20±7	21±5
2	8–11							15±4*	25±10
8	3– 4	132±5	139±2***	800±19	865±60****	22±1	23±2*		
10	4	127±6	134±4***	789±50	828±32****	22±1	23±1*	17±2	17±4
12	4– 6	127±2	132±5***	776±18	829±42****	21±1	22±1*	29±5*	17±5
Male									
−1	11–15							15±6	21±6
2	8–11							14±2	17±3
8	4	134±6	134±4	793±66	804±24	22±1	22±1		
10	4	130±3	130±7	783±37	803±73	22±1	21±1	16±2	16±3
12	5– 6	132±5	132±3	746±11	788±11	22±1	22±1	32±9 ****	17±3

* $p < 0.025$; ** $p < 0.01$; *** $p < 0.005$; **** $p < 0.001$.

lar for both control and castrated male rats. The calcium/hydroxyproline ratios were close to 36, and no differences were observed between any of the groups.

Mechanics. The strength of the femora was not significantly influenced by castration in either sex. The ultimate torsional moment in female and male rats was about 0.4 Nm and 0.7 Nm respectively.

Calcitonin. The plasma levels of iCT were decreased at 2 weeks after castration in female rats when compared to both controls ($P < 0.025$) and precastration levels ($P < 0.02$). High iCT levels were however, observed in castrated female and male groups compared to their controls 12 weeks after the operation ($P < 0.01$) (Table 2).

Discussion

Our study confirmed that middle-aged female rats on a normal diet develop osteoporosis after castration; similar findings have been made by Saville (1969) and Kalu (1983). An increased weight of castrated female rats compared to controls has also been recorded by Saville (1969) and Langeland (1975b). The latter found that the collagen metabolism was higher in castrated than in normal female rats. This may be explained by lack of anticatabolic oestrogens. Lack of anabolic testosterones did not seem to influence body weight and bone density of male rats in our study. This may indicate that the general metabolism is more influenced by castration in female than in male rats. The age variation between the two sexes is probably of minor importance as Wistar rats are fully grown at the age of 5–6 months.

Both the bone density and the calcium and hydroxyproline concentrations of the bones were reduced in the female castrated rats. The calcium/hydroxyproline ratio was, however, unchanged. This agrees with the findings in human osteoporotic bone (Johnson & Epstein 1982).

CT is a bone-preserving hormone: it inhibits bone resorption (Copp 1979). The circulating levels of iCT are influenced by the sex hormones, and high levels of the hormone are found during pregnancy and after administration of the oral contraceptive pill. In contrast, the iCT levels have been reported to be lower in postmenopausal than in normal women (Heath & Sizemore 1977). The circulating iCT levels have also been reported to be lower in postmenopausal women with osteoporosis than in those without (Milhaud et al. 1978, Jensen et al. 1984). In our study, iCT was decreased during the first weeks after ovariectomy. This agrees with the findings of Cressent (1981) and Kalu (1983). A reduced ovarial function may therefore transiently reduce the circulating iCT levels in rats. Whether this has any influence on the development of osteoporosis in these rats is at present unknown.

We conclude that adult female, unlike male rats, kept on a normal diet develop osteoporosis after castration. Female castrated rats may therefore be useful for studying post-menopausal osteoporosis.

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