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Role of Parathyroid Hormone and 1,25-Dihydroxyvitamin D_3 in the Development of Osteopenia in Oophorectomized Rats

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Summary. The effect of ovarian insufficiency on calcium metabolism has been thought to involve an increased bone resorptive effect of parathyroid hormone and possible impaired synthesis of 1,25-dihydroxyvitamin D_3. In the present study a rat model allowing for controlled serum levels of parathyroid hormone and 1,25-dihydroxyvitamin D_3 was used. Oophorectomy in this species is associated with increased serum levels of 1,25-dihydroxyvitamin D_3 and decreased bone mass. Although thyroparathyroidectomy increased bone mass, an increased sensitivity of bone to parathyroid hormone in oophorectomized rats was not observed. Thus the development of the osteopenia did not seem to be related to increased parathyroid hormone sensitivity or to reduced levels of 1,25-dihydroxyvitamin D_3. Exogenous 1,25-dihydroxyvitamin D_3 increased bone mass in oophorectomized as well as intact rats. Intestinal calcium transport was increased by moderate doses of 1,25-dihydroxyvitamin D_3. Intestinal calcium transport was also reduced by thyroparathyroidectomy and increased by the administration of parathyroid extract. A tendency for increased accumulation of 1,25-dihydroxyvitamin D_3 in blood in oophorectomized rats has been noted. It is suggested that the tendency to hypercalcemia in ovarian-insufficient females given 1-hydroxylated vitamin D compounds may be related to a diminished metabolism of 1,25-dihydroxyvitamin D_3.

Key words: Rats — Osteopenia of oophorectomy — 1,25-Dihydroxyvitamin D_3 — Parathyroid hormone — Osteoporosis.

Oophorectomy in rats has been used as a model for postmenopausal osteoporosis [1, 2]. Like that in humans, oophorectomy in rats appears to result in an increased tendency for bone resorption thought to be caused by increased sensitivity of bone to parathyroid hormone (PTH) [3–6]. This could then lead to a depressed serum level of PTH resulting in low 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$] levels and a shift of the source of calcium from the intestine to the bone [7]. It might be expected therefore that exogenous 1,25$(OH)_2D_3$ could improve calcium balance in the postmenopausal state. The present study was undertaken to study this in a rat model of sex hormone deficit osteoporosis in which defined conditions regarding PTH and 1,25$(OH)_2D_3$ could be created.

Materials and Methods

For this study 100 four-month-old female Holtzman rats (Madison, WI) were used. Their mean initial body weight was 255 g. One week before the start of the experiment 50 rats chosen at random were oophorectomized (OOX); 20 of the OOX rats and 20 intact rats had been subjected to thyroparathyroidectomy (TPT-X). The remaining rats were subjected to sham operations. All operations were performed under ether anesthesia. From weaning to 1 week before the experiment the rats were fed a stock diet containing 1.2% calcium, 0.3% phosphorus, and adequate amounts of vitamin D. They were divided into the following groups:

Intact control	OOX
Intact + 1,25-$(OH)_2D_3$	OOX + 1,25-$(OH)_2D_3$
Intact + high dose of 1,25-$(OH)_2D_3$	OOX + high dose of 1,25-$(OH)_2D_3$
Intact + TPT-X	OOX + TPT-X
Intact + TPT-X + parathyroid extract (PTE)	OOX + TPT-X + PTE

The rats were kept in individual cages during the experimental period of 10 weeks. They were given distilled water and a diet

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[1] Abbreviations used: PTH, parathyroid hormone; 1,25$(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; OOX, oophorectomized; TPT-X, thyroparathyroidectomized; PTE, parathyroid extract; 25OHD_3, 25-hydroxyvitamin D_3; 24,25$(OH)_2D_3$, 24,25-dihydroxyvitamin D_3.

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containing 0.5% Ca and 0.3% P ad libitum [8]. Three times a week 20 IU of vitamin D_3 was added to the food, and in addition TPT-X rats received 7 μg of synthetic L-thyroxin per day. This dose of vitamin D is known to be adequate for rats [9, 10]. Only rats having a serum calcium of less than 8 mg/100 ml under these circumstances were considered to have undergone parathyroidectomy. Bovine PTH extract dissolved in 10 mM acetic acid was injected daily at a dose of 25 U; this dose had to be increased to 50 U/day to maintain an increase of serum calcium of 1 mg/100 ml or more at 6 h after the injection. $1,25(OH)_2D_3$ was dissolved in propylene glycol (0.1 ml) and injected subcutaneously 3 times a week at a dose of 75 or 150 ng. These doses may be pharmacological since it is believed that a 60 kg man may synthesize about 1–2 μg/day. The rats were killed by bleeding from the aorta under ether anesthesia, and 3–5 ml of serum could be obtained from each rat. Duplicate determinations of serum calcium using atomic absorption spectrometry in the presence of 1% lanthanum chloride and of serum phosphorus using the method of Chen et al. [11] were carried out. Determination of 25-hydroxyvitamin D_3 ($25OHD_3$), $1,25(OH)_2D_3$, and 24,25-dihydroxyvitamin D_3 [$24,25(OH)_2D_3$] were done according to Shepard et al. [12].

Intestinal calcium transport was measured using everted intestinal sacs as described by Martin and DeLuca [13], but the ratio of absolute calcium concentrations was used instead of the ^{45}Ca ratio. Bone analyses were carried out using the right femurs which were stored at −20°C. They were then thawed, dissected free of soft tissue, and put in tubes with distilled water for 4–6 h. The weight of the bone in water and in air was recorded and the volume was calculated according to Archimedes' principle. The bones were then mounted on a sheet of paper, which was placed over an x-ray film, and roentgenograms were obtained. The length of the bone, the inner and outer diameter at the mid-point were measured from the x-ray pictures with a magnifier provided with a microscale. The bones were then dried and defatted in a Soxhlet extractor for 48 h first with ethanol, then with ether. They were subsequently dried at 80°C for 24 h and weighed. The bones were ashed at 500°C for 48 h, kept in a desiccator for at least 40 h, and weighed. Student's t test was used for the statistical evaluation. Crystalline $1,25(OH)_2D_3$ and $25OHD_3$ was provided by Dr. M. Uskokovic, Hoffmann-La Roche, Inc. (Nutley, NJ); parathyroid extract was supplied by the Eli Lilly Co. (Indianapolis, IN).

Results

Seven rats died during the operative procedures, and two rats died for reasons unrelated to the experiment. Four TPT-X rats did not have a serum Ca level below 8 mg/100 ml and therefore were not regarded as having undergone a complete thyroparathyroidectomy. None of these rats was included in the study.

All rats increased in body weight. The mean final body weight of the OOX rats was significantly higher than the weight of the corresponding sham-operated controls (Table 1). As expected, TPT-X reduced the weight gain which was restored by PTH. $1,25(OH)_2D_3$ at the low dose increased body weight. Serum Ca rose significantly in all rats given $1,25(OH)_2D_3$ and significantly more in the groups receiving the high dose (Table 2). Serum Ca increased to a greater degree in OOX rats compared to intact rats as a result of treatment with the high dose of $1,25(OH)_2D_3$, whereas a significant increase in response to the low dose of $1,25(OH)_2D_3$ was found only in the OOX rats. This was not seen initially but appeared by the second week of treatment. This rise in serum Ca was present after more than 24 h fasting, although the values became somewhat lower. OOX did not affect serum Ca in TPT-X rats and after PTE administration. Serum P was not altered by OOX or by $1,25(OH)_2D_3$ treatment. As expected, TPT-X increased serum P. PTH reduced serum P in rats not having undergone OOX.

There was, as expected in adult rats, low Ca transport in the control groups (Table 3). In the TPT-X groups, a low active transport was seen, whereas a significantly increased Ca transport occurred in animals given $1,25(OH)_2D_3$. There was no further increase in Ca transport with the high dose of $1,25(OH)_2D_3$. In all conditions except TPT-X there was a lower intestinal Ca transport after OOX than in intact animals but the differences were not significant. There were no differences in $25OHD_3$ levels between OOX and intact animals. Plasma $1,25(OH)_2D_3$ levels in all cases were higher in OOX rats compared to intact rats. This difference was statistically significant in the groups dosed with $1,25(OH)_2D_3$ (Table 4). The $24,25(OH)_2D_3$ values were slightly higher in the

Table 1. Effects of oophorectomy on body weights of rats following a variety of treatments

	Control $N = (10),10$	TPT-X $N = (8),10$	TPT-X + PTE $N = (7), 7$	+$1,25(OH)_2D_3$ $N = (10),9$	+high $1,25(OH)_2D_3$ $N = (10),9$
Initial body weight (g)	(251 ± 10) 255 ± 12	(257 ± 10) 255 ± 9	(256 ± 9) 255 ± 8	(258 ± 6) 252 ± 12	(259 ± 11) 254 ± 12
Final body weight (g)	(352 ± 35) 308 ± 17[a]	(326 ± 15) 283 ± 22[b]	(350 ± 13) 300 ± 18[b]	(381 ± 24) 324 ± 12[b]	(343 ± 27) 288 ± 15[c]

Values from OOX rats in parentheses. P values are for the differences between control and OOX rats. Mean ± SE

[a] $0.01 < P < 0.05$

[b] $0.001 < P < 0.01$

[c] $P < 0.001$

Table 2. Serum calcium and phosphorus levels

	Control	TPT-X	TPT-X + PTE	+1,25$(OH)_2D_3$	+high 1,25$(OH)_2D_3$
Serum Ca	(10.0 ± 0.2)	(6.1 ± 0.7)	(8.2 ± 0.9)	(11.4 ± 0.7)	(12.4 ± 0.5)
(mg/100 ml)	10.2 ± 0.1	6.3 ± 1.0	8.2 ± 1.0	10.6 ± 0.7[b]	11.5 ± 0.6[b]
Serum P	(6.1 ± 0.8)	(8.0 ± 1.0)	(8.7 ± 0.6)	(6.9 ± 0.9)	(6.9 ± 0.6)
(Mg/100 ml)	6.5 ± 0.7	7.9 ± 0.7	6.8 ± 1.0[a]	6.5 ± 0.9	6.8 ± 0.6

Values from OOX rats in parentheses. P values are for comparison between OOX and control rats. Mean ± SE
[a] $0.005 < P < 0.01$
[b] $0.001 < P < 0.01$

Table 3. Intestinal calcium transport

Control	TPT-X	TPT-X + PTE	+1,25$(OH)_2D_3$	+high 1,25$(OH)_2D_3$
(2.2 ± 0.9)	(1.9 ± 0.4)	(2.5 ± 0.4)	(3.5 ± 0.7)[b]	(3.5 ± 0.4)
2.8 ± 0.4	1.6 ± 0.3	2.8 ± 0.6[a]	4.0 ± 0.4[b]	3.8 ± 0.2

Values from OOX rats in parentheses
Values are expressed as the final ratio of Ca in the serosal medium to that in the mucosal. Mean ± SE
[a] Significantly different from TPT-X, $P < 0.001$
[b] Significantly different from control, $P < 0.001$

Table 4. Vitamin D metabolite levels

Treatment	Control	TPT-X	TPT-X + PTE	+1,25$(OH)_2D_3$	+high 1,25$(OH)_2D_3$
N	(5)	(5)	(5)	(5)	(5)
	5	5	5	4	4
25OHD_3	(5.1 ± 0.4)	(3.9 ± 0.7)	(2.9 ± 0.4)	(3.2 ± 0.2)	(3.5 ± 2.2)
ng/ml	5.1 ± 0.4	2.9 ± 0.2	3.5 ± 0.3	3.1 ± 0.3	3.1 ± 1.9
1,25$(OH)_2D_3$	(26.0 ± 6.8)	(11.7 ± 3.0)	(17.4 ± 5.1)	(54.4 ± 6.4)	(580.3 ± 229.0)
pg/ml			(n = 4)		
	14.6 ± 1.7[a]	<2.0[b]	13.8 ± 5.9	28.8 ± 6.0[b]	395.2 ± 115.1
24,25$(OH)_2D_3$	(8.4 ± 0.9)	(3.1 ± 0.4)	(4.8 ± 0.8)	(3.4 ± 0.5)	(3.4 ± 0.9)
ng/ml)	9.9 ± 2.2	2.9 ± 0.2	3.5 ± 0.9	2.9 ± 0.3	2.8 ± 0.8

Values from OOX rats in parentheses. P values are for comparison of OOX v sham-operated control. Mean ± SE
[a] $0.01 < P < 0.05$
[b] $0.001 < P < 0.01$

OOX rats compared to corresponding intact rats except for the controls (Table 4).

The bone measurements revealed no differences in bone length between the groups (Table 5). Further, bone volume was not significantly changed by the treatments. Even the OOX rats with their larger body weight had the same femur volume as controls. The percent ash showed no significant change that would indicate a disturbance of mineralization; but a small difference was found between the OOX and intact rats treated with 1,25$(OH)_2D_3$. The decrease in the amount of mineral per volume of bone reflects the osteopenia. A significant decrease in bone density resulting from OOX was found in all circumstances. Furthermore, TPT-X and 1,25$(OH)_2D_3$ administration increased the ash/volume ratio in both the OOX and intact rats. The high 1,25$(OH)_2D_3$ dose, however, decreased this value in the OOX rats whereas this was not seen in the intact rats. The total ash values seemed to confirm the above observations, but due to the individual variations in femur size the differences were not statistically significant.

Discussion

Oophorectomy led to the expected weight gain in rats. It also resulted in a highly significant decrease in mineral/volume of the femur, analogous to the postmenopausal state in man. Thyroparathyroidectomy did not prevent the effect of OOX on bone density, but it did give higher values as expected. These results would argue that the osteopenia resulting from lack of sex hormones is not the result of increased sensitivity to parathyroid hormone. This view is supported by the fact that treatment of TPT-X rats with PTE did not increase the

Table 5. Characteristics of bone from oophorectomized rats given $1,25(OH)_2D_3$ or parathyroid hormone

	Control	TPT-X	TPT-X + PTE	$+1,25(OH)_2D_3$	+high $1,25(OH)_2D_3$
Length	(37 ± 1)	(38 ± 1)	(37 ± 1)	(38 ± 1)	(38 ± 1)
(mm)	36 ± 1	36 ± 1	37 ± 1	38 ± 1	37 ± 1
Total ash	(358.8 ± 35.9)	(386.1 ± 17.6)	(367.7 ± 22.1)	(377.5 ± 30.3)	(344.6 ± 30.9)
(mg)	377.5 ± 29.7	375.8 ± 32.0	367.6 ± 19.8	399.1 ± 34.4	369.9 ± 48.7
Ash weight/	(646.7 ± 25.3)	(701.2 ± 21.5)	(670.6 ± 49.5)	(675.9 ± 36.7)	(607.7 ± 47.2)
volume (mg/cm^3)	697.6 ± 23.8[b]	746.1 ± 29.7[a]	764.3 ± 55.3[a]	761.6 ± 62.3[a]	727.3 ± 69.6[b]
Ash as percent	(67.9 ± 1.3)	(70.7 ± 0.7)	(69.2 ± 1.6)	(68.8 ± 0.9)	(68.3 ± 1.3)
of dry weight (%)	68.2 ± 3.3	71.2 ± 1.0	69.9 ± 1.3	70.3 ± 1.3[a]	69.6 ± 1.3

Values from OOX rats in parentheses. P values are for comparison between OOX and sham-operated controls. Mean ± SE
[a] $0.001 < P < 0.01$
[b] $P < 0.001$

osteopenia of OOX. This is in contrast to the report of Orimo et al. [4] in which the bone loss resulting from PTE administration was increased by OOX. However, the bone loss observed in that experiment may have been the result of oophorectomy alone, since that control was not included. It is also in contrast to the conclusion drawn by Atkins et al. [5] that PTE is more effective in causing bone resorption in the absence than in the presence of estrogen. This was based on in vitro studies involving super physiologic concentrations of PTE. It is important to note that TPT-X decreased serum Ca, increased serum P, and decreased serum $1,25(OH)_2D_3$. PTH increased serum Ca, decreased serum P, and increased $1,25(OH)_2D_3$ levels illustrating the effectiveness of these manipulations.

Intestinal Ca transport, measured by an in vitro technique, was not significantly reduced by OOX. This agrees with the measurements of plasma $1,25(OH)_2D_3$ levels (Table 4). This differs from the results in man [7, 12] that may suggest a species difference or that the transport measurements here do not necessarily represent overall intestinal absorption. It is of interest that both PTE and $1,25(OH)_2D_3$ increased intestinal Ca transport (Table 3).

Hypercalcemia was found to parallel the serum level of $1,25(OH)_2D_3$ in rats given exogenous $1,25(OH)_2D_3$. Since hypercalcemia did not develop as a result of these doses until after 2 weeks of treatment, it is possible that $1,25(OH)_2D_3$ accumulates under these circumstances. This is supported by the fact that doubling a dose of $1,25(OH)_2D_3$ increased the serum level of $1,25(OH)_2D_3$ 10-fold. Furthermore, the level of $1,25(OH)_2D_3$ in serum is higher in OOX rats as a result of endogenous production of $1,25(OH)_2D_3$.

To eliminate the variable role of the parathyroid gland, parathyroidectomy followed by administration of a constant amount of PTH was used. As expected, TPT-X reduced serum $1,25(OH)_2D_3$ level while PTH increased it [14]. On the other hand, it has been suggested that sex hormones stimulate 25OHD-1α-hydroxylase [15]. If this is the case in the rat, there must be an overriding effect of gonadal hormones on metabolism and excretion of $1,25(OH)_2D_3$.

The present findings may explain Marshall's observation that hypercalciuria during treatment with 1α-OHD_3 occurs more frequently in the treatment of postmenopausal women [16]. This may be caused by accumulation of $1,25(OH)_2D_3$ rather than by increased sensitivity of bone to $1,25(OH)_2D_3$. In spite of the risk of hypercalcemia, it must be emphasized that the depression of intestinal absorption of Ca in oophorectomy can be overcome by the administration of low doses of $1,25(OH)_2D_3$; the PTH level may decrease and further resorption of bone be prevented. With lower doses of $1,25(OH)_2D_3$ bone mineral content is increased in both OOX and intact rats. This confirms earlier findings that $1,25(OH)_2D_3$ can counteract demineralization after oophorectomy in rats [2]. However, the *high dose* of $1,25(OH)_2D_3$ resulted in hypercalcemia and diminished bone mass, which suggests that a careful adjustment of dose is required to have the desired effect on bone mass without accompanying hypercalcemia.

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