Influences of Sex Hormone on Remodeling of the Mandibular Condyle in Mice

Ph. D. Thesis

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1. Introduction

1.1 Review of the literature

Osteoporosis with aging or after the menopause in women is characterized by an enhanced bone turnover associated with more excessive bone resorption than bone formation which is essentially due to reduced estrogen levels (Ohta *et al.* 1992; Riggs 1991). Such phenomenon may be explained by the fact that estrogen deficiency increases bone turnover, or more substantial resorption and less formation, resulting in a net loss of bone mineral density, primarily in the trabecular compartment (Riggs 1991). On the other hand, hypogonadism is also recognized as a risk factor for osteoporosis in men (Anderson 1992); *i.e.* the lack of androgen in men causes excessive bone resorption similarly to osteoporosis in women (Anderson 1992; Devogelaer *et al.* 1992; Finkelstein *et al.* 1987).

Furthermore, enhancement of B lymphopoiesis was found in bone marrow of orchiectomized (ORX) and ovariectomized (OVX) mice (Wilson *et al.* 1995). Cancellous and cortical bone density decreases according to the degree of disorders associated with hypogonadism (Finkelstein *et al.* 1989; Greenspan *et al.* 1986), which occasionally prevails in male patients hospitalized for immobile sickness such as vertebral crush fracture (Seeman *et al.* 1983). Androgens stimulate normal skeletal development during puberty (Johansen *et al.* 1988), and the delay in puberty in humans is associated with a lower peak bone mass (Finkelstein *et al.* 1992). The onset of osteoporosis, therefore, depends on both peak bone mass and bone loss after maturation (Riggs and Melton 1986). However, the mechanism of osteoporosis in male has not been elucidated.

In transgenic estrogen receptor knockout mice used to examine a role of estrogen in the maintenance of bone architecture, remarkable decrease in the trabecular

bone volume was induced similarly in both the male and female (Korach 1994). Furthermore, serious osteoporosis was found in male subjects with an estrogen resistance (Smith *et al.* 1994). It has recently been reported as the effect of testosterone on bone cells that aromatase (P450arom) alters testosterone into estrogen (E2) which further affects bone cells (Tanaka *et al.* 1993), indicating that estrogen may be important for bone metabolism in both male and female. However, it is unclear how estrogen and androgen influence bone metabolism.

Prevalence of temporomandibular joint disorders (TMDs) has recently increased in growing and young adult populations. It is clinically recognized that the prevalence of TMDs is higher in female than in male. In etiologic studies, it was reported that approximately 20% (Heloe and Heloe 1979) to 40% (Helkimo 1979) of general population had a past or present history of such dysfunction, and the prevalence was eight (Farman *et al.* 1982) to ten times (Aufdemorte *et al.* 1986) higher in female than in male. Furthermore, TMDs were found in 68% of adolescents, and the prevalence was higher in girls than in boys (Grosfeld *et al.* 1985). Therefore, it may be assumed that the prevalence of TMDs is related with sex hormone, estrogen of women in particular.

The nature of TMDs in adolescents is revealed as internal derangement of the TMJ, defined as an abnormal positional and functional relationship between the articular disk, the mandibular condyle and the glenoid fossa. Condylar resorption, regarded as a progressed form of the internal derangement, is demonstrated to alter the growth pattern of the craniofacial skeleton, the mandible in particular. Condylar remodeling and the subsequent mandibular growth, meanwhile, are of great importance to achieve normal jaw function associated with harmonious craniofacial morphology.

OVX and ORX are well understood to affect bone remodeling in terms of enhanced bone turnover. Thus, these seem great significant experimental models to examine the mechanisms of sex hormone on bone remodeling. Mandibular condyle is composed of a secondary or embryonic type of cartilage, different in the origin and histological organization from the epiphyseal growth plate of long bones (Dibbets 1990; Durkin 1972). It is thus of great interest to elucidate how OVX and ORX affect the remodeling of mandibular condyle and whether or not the injection of E2 and DHT exerts different effects. However, the effects of E2 and non-aromatizable DHT on condylar remodeling are still unclear.

1.2 Objectives

This study was designed to examine the influence of sex hormone on bone remodeling in the condylar head, in terms of the number of TRAP-positive cells and the amount of trabecular bone volume in the OVX and ORX mice subjected to injection of estrogen and androgen.

2. Materials and Methods

2.1 Experimental animals and the treatments

One hundred and seventy 8-week-old C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME, USA) were used in this experiment.

Table 1. Summary of experimental animals

	Experimental period			
Group	2W	4W	8W	12W
OVX	5	5	5	5
ORX	5	5	5	5
OVX+E2	5	5	5	5
ORX+E2	5	5	5	5
OVX+DHT	5	5	5	5
ORX+DHT	5	5	5	5
Cont. F	5	5	5	5
Cont. M	5	5	5	5

(Number)

Under general anesthesia with sodium pentobarbital, male and female mice underwent ovariectomy (OVX) and orchiectomy (ORX) at 8-week-old, respectively. In each of the experimental groups, five mice were sacrificed 2, 4, 8 and 12 weeks after surgery for OVX and ORX (Table 1). Estrogen and androgen were given daily immediately after the surgery by subcutaneous injection of 17ß-estradiol (E2, 10μg/kg; Research Biochemicals International, Natick, MA, USA) and 5∂-dihydrotestosterone (DHT, 100μg/kg; Fluka, Milwaukee, WI, USA) which were dissolved in 5% benzyl alcohol (Katayama Chemical Industries Co., Osaka, Japan) and 95% corn oil (Katayama Chemical Industries Co., Osaka, Japan)

(Gallagher *et al.* 1996; Sato *et al.* 1993). These mice were divided equally into six experimental groups with OVX, ORX, OVX+E2, ORX+E2, OVX+DHT, ORX+DHT and untreated male and female control groups. The body weight was measured every four days.

2.2 Histological examination

The mandibular condyles were fixed with 4% formaldehyde, decalcified in EDTA (pH 7.4) for two weeks, dehydrated in an ascending ethanol series (70, 80, 90, 95, 99, 100%), embedded in paraffin and cut into frontal sections of 7μm thickness. The sections were stained with tartrate-resistant acid phosphatase (TRAP) and azocarmine-aniline blue (AZAN) and observed by an optical microscope (BH2-RFCA, Olympus Optical Co., Tokyo, Japan). The TRAP stained sections were used to count the number of osteoclasts in the condylar head.

2.3 Histomorphometric analysis

The sections stained with AZAN were used for the histomorphometric analyses. Bone histomorphometric analyses were performed in the subchondral area of the condyle, by use of an image analysis program of NIH Image 1.59 (National Institutes of Health, Bethesda, MD, USA). On the sections passing through the center of mandibular condyle, the number of TRAP-positive cells was counted. For the trabecular bone volume, the area was measured on the frontal sections and then the means were calculated.

2.4 Statistical treatment

An one-way analysis of variances (ANOVA) was executed to examine

differences in the variances between groups. Then, pairwise comparisons (Fisher) were performed to examine the differences between groups.

3. Results

3.1 Changes in the body weight

No remarkable differences in the body weight were found between the OVX or ORX mice and the corresponding controls (Figure 1). Thus, the experimental mice exhibited almost similar general growth to the controls, indicating negligible influences of the surgery on general growth in terms of the changes in body weight.

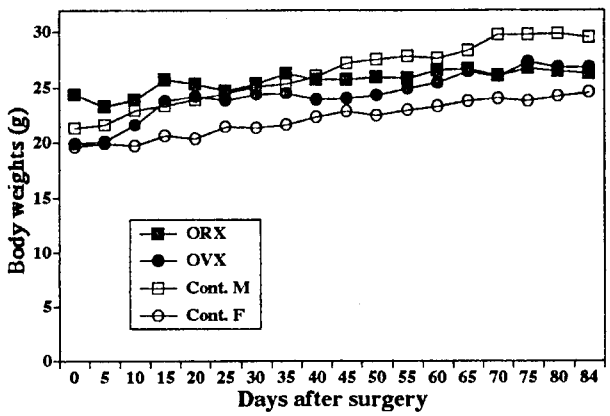

Figure 1. Changes in body weights in the OVX, ORX and control groups.

In the OVX and ORX mice with E2 injection, remarkable increases in the body weights were found in comparison with the uninjected OVX and ORX mice (Figure 2). In the OVX and ORX mice treated with DHT, substantial increases in the body weights were similarly recognized, if compared with the untreated OVX and ORX mice (Figure 3). These findings demonstrate substantial effects of E2 and DHT on the increase in body weight or general growth.

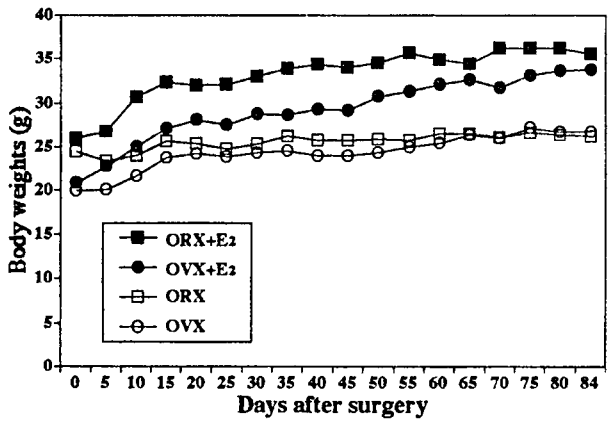

Figure 2. Changes in body weights in the OVX and ORX mice with E2 injection and untreated OVX and ORX groups.

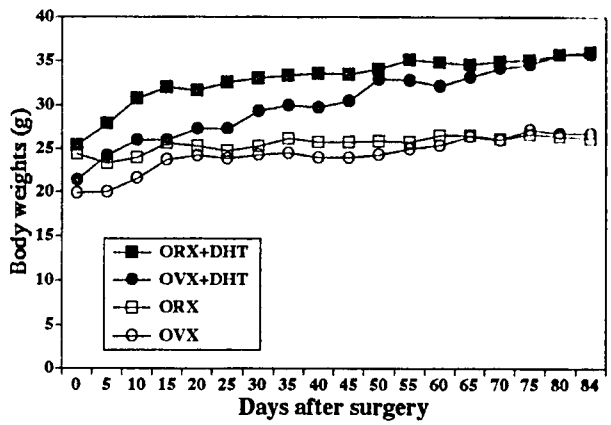

Figure 3. Changes in body weights in the OVX and ORX mice with DHT injection and untreated OVX and ORX groups.

Changes in the body weights are shown in Figures 4 and 5 for OVX and ORX mice with injection of E2 and DHT. No significant differences in the body weights were found between the OVX mice injected with E2 and DHT (Figure 4). For the ORX mice with the injection, no significant differences were similarly observed for the entire experimental period (Figure 5). From these results, it is indicated that the effects of E2 and DHT on general growth are almost similar in terms of the body weight.

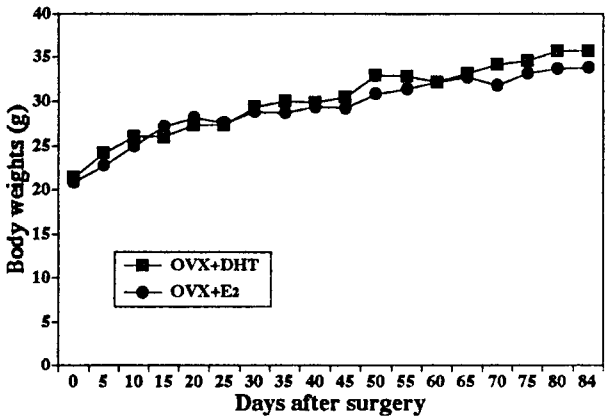

Figure 4. Changes in body weights in the OVX mice with injection of E2 and DHT.

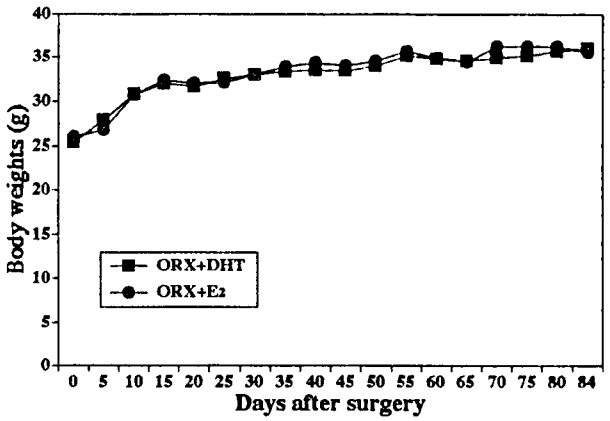

Figure 5. Changes in body weights in the ORX mice with injection of E2 and DHT.

3.2 Histological and histomorphometric changes

3.2.1 Changes incident to OVX and ORX

Figure 6 shows changes in the number of TRAP-positive cells in the mandibular condyles of the OVX and ORX mice and the controls. In the female control group, TRAP-positive cells exhibited a gradual decrease in the number from 2 to 12 weeks after the surgery, although the decrease was considerable from 2 to 4 weeks postsurgery. OVX mice, meanwhile, exhibited the most prominent increase in the number of TRAP-positive cells 4 weeks after surgery, and a gradual decrease at the following experimental stages. Significant differences in the number were found between the OVX and female control mice at 4, 8 and 12 weeks after the surgery (Figure 6). TRAP-positive cells in the male control mice also exhibited a gradual decrease similar to the female controls, although the changes were more invariable than in the female control mice. On the other hand, ORX mice exhibited the most substantial increase in the number of TRAP-positive cells 4 weeks after surgery, and tended to approach the control levels more approximately than OVX mice.

Significant differences in the number were also found between the ORX and male control groups at 4 and 8 weeks after the surgery (Figure 6). Furthermore, the changes were less in the ORX mice than in the OVX group.

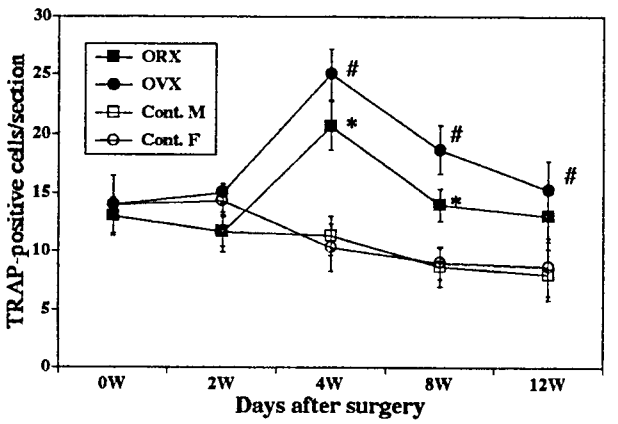

Figure 6. Changes in the number of TRAP-positive cells/section of the condylar head in the OVX, ORX and control groups. *P<0.05 vs. male control mice, #P<0.05 vs. female control mice.

Trabecular bone volume in the condylar head of the female control mice increased gradually from 2 to 12 weeks after the surgery, although the changes from 2 to 8 weeks postsurgery were the most substantial. On the contrary, the OVX mice exhibited a gradual decrease in the volume with significant differences from the controls for the period of 4 to 12 weeks after the surgery (Figure 7). In the male controls, the trabecular bone volume similarly increased to the female group, although the changes were smaller than the female controls. The trabecular bone volume was significantly less in the ORX mice than in the controls for the experimental period of 4 to 8 weeks after the surgery. However, the changes were

less in the ORX group than in the OVX mice (Figure 7).

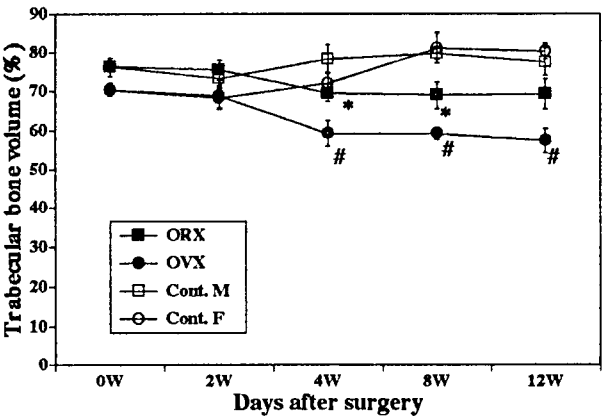

Figure 7. Changes in the trabecular bone volume of the condylar head in the OVX, ORX and control groups.
*P<0.05 vs. male control mice, #P<0.05 vs. female control mice.

These histomorphometric findings indicate that the most prominent decrease in the trabecular bone volume is induced for both OVX and ORX mice 4 weeks postsurgery. The actual structures of the condyles are shown in Figure 8. Trabecular bone loss was more apparent in the OVX and ORX mice 4 weeks after surgery than in the corresponding controls.

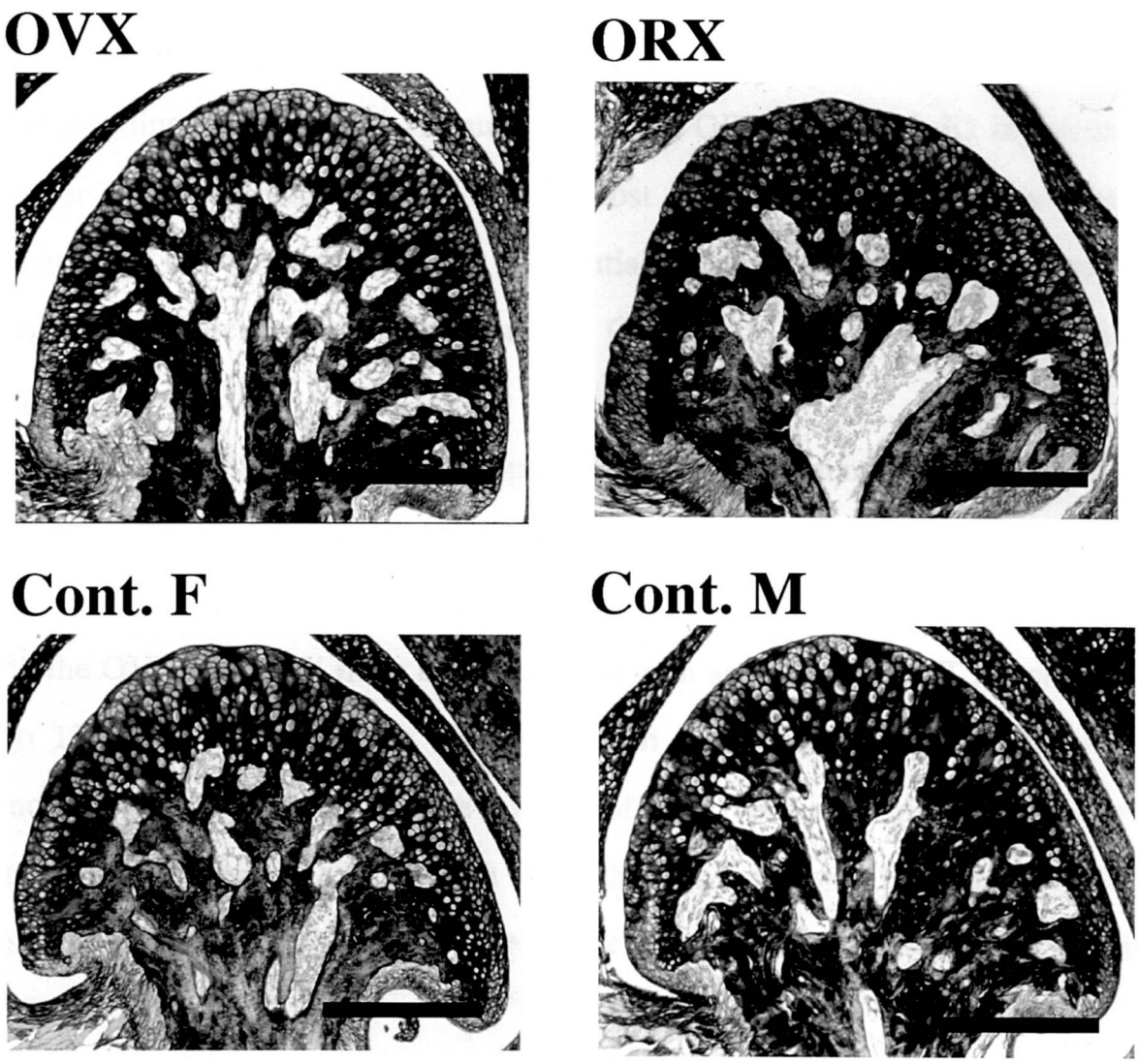

Figure 8. Photomicrographs of the condyles in the OVX and ORX mice and the controls 4 weeks after surgery. Bars denote 500 μm. (AZAN staining; $\times$100)

3.2.2 Changes incident to injection of E2 and DHT

Figures 9 and 10 show changes in the number of TRAP-positive cells in the mandibular condyle of the OVX and ORX mice with E2 injection. Decrease in the number of TRAP-positive cells was initiated at 2 weeks up to 12 weeks after surgery in the OVX mice with E2 injection. The number was significantly larger if compared with the uninjected OVX, approaching the numbers in the corresponding control group (Figure 9). For the ORX mice with E2 injection, the number of TRAP-positive cells were almost invariable until 4 weeks after the surgery and thereafter exhibited a substantial decrease up to 12 weeks, with the significant differences from the untreated ORX mice at 4, 8, 12 weeks after the surgery (Figure 10).

Changes in the number of TRAP-positive cells in the mandibular condyle were shown in Figures 11 and 12 for the OVX and ORX mice with DHT injection. Significant differences in the number of the TRAP-positive cells were found for both the OVX and ORX mice between those with and without DHT injection from 4 to 12 weeks after surgery. The changes in the mice with the injection were almost similar to the controls. The number of TRAP-positive cells decreased more significantly in the ORX mice with DHT than in those with E2, whereas TRAP-positive cells in the OVX mice were more substantially suppressed by E2 than DHT.

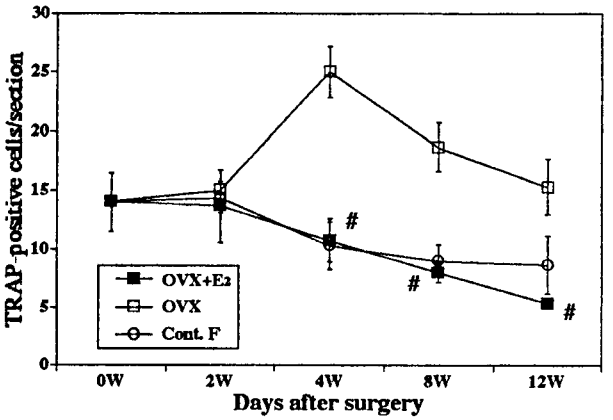

Figure 9. Changes in the number of TRAP-positive cells/section in the OVX mice with and without E2 injection, and the female controls. #P<0.05 vs. OVX mice.

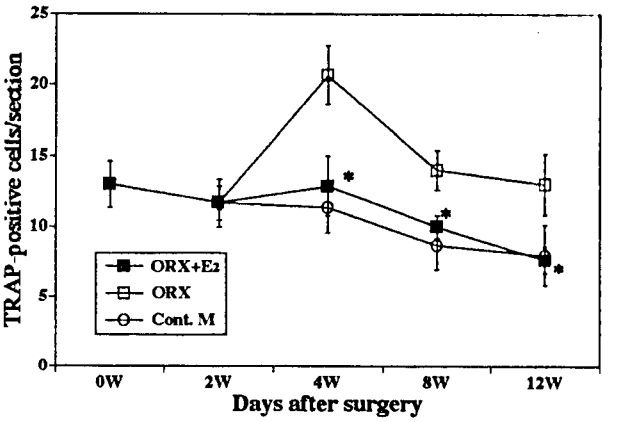

Figure 10. Changes in the number of TRAP-positive cells/section in the ORX mice with and without E2 injection, and the male controls. *P<0.05 vs. ORX mice.

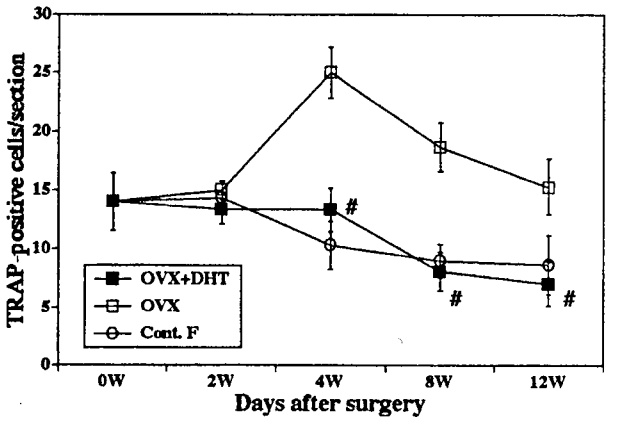

Figure 11. Changes in the number of TRAP-positive cells/section in the OVX mice with and without DHT injection, and the female controls. #P<0.05 vs. OVX mice.

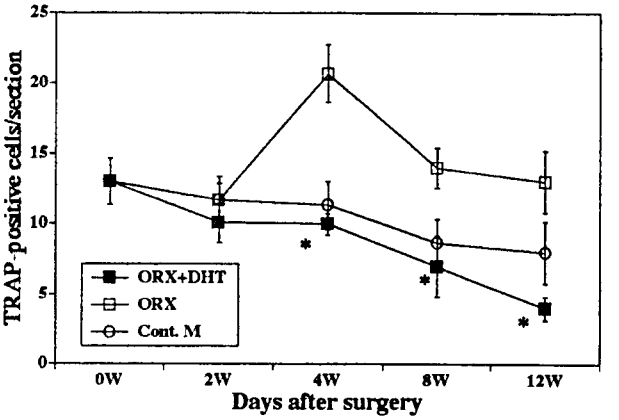

Figure 12. Changes in the number of TRAP-positive cells/section in the ORX mice with and without DHT injection, and the male controls. *P<0.05 vs. ORX mice.

Figures 13 and 14 show changes in the trabecular bone volume in the mandibular condyle of the OVX and ORX mice with E2 injection. Following the decrease in TRAP-positive cells described above, the trabecular bone volume became larger in the OVX and ORX mice with E2 injection from 4 to 12 weeks after surgery than in the non-treatment OVX and ORX mice. Changes in the trabecular bone volume in the mandibular condyle are shown for the OVX and ORX mice with DHT injection

(Figures 15 and 16). Trabecular bone volume became significantly larger only 12 weeks after surgery in the OVX and ORX mice with DHT injection than in the non-treatment OVX and ORX mice, although the decrease in the number of TRAP-positive cells was initiated 4 weeks after surgery and lasted until the end of experiment.

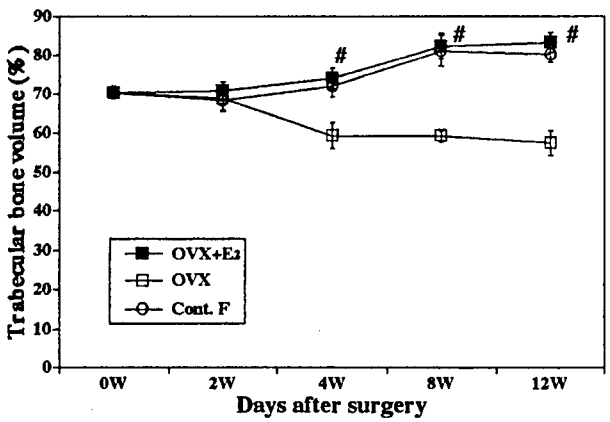

Figure 13. Changes in the trabecular bone volume in the OVX mice with and without E2 injection, and the female controls. #P<0.05 vs. OVX mice.

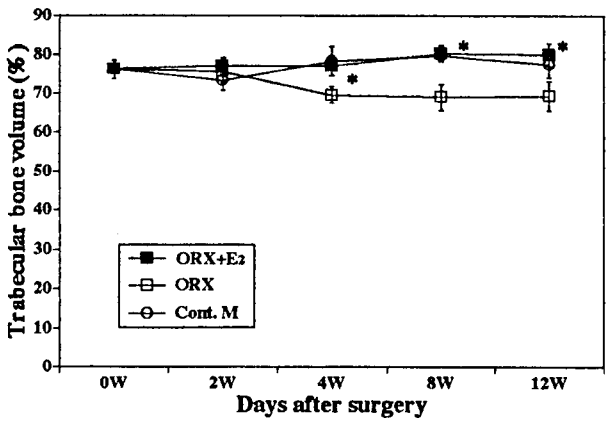

Figure 14. Changes in the trabecular bone volume in the ORX mice with and without E2 injection, and the male controls. *P<0.05 vs. ORX mice.

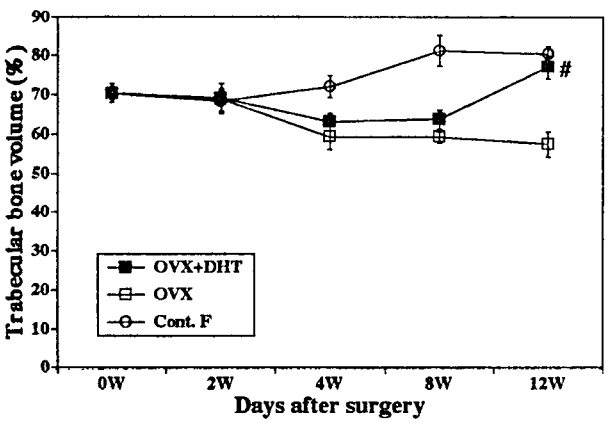

Figure 15. Changes in the trabecular bone volume in the OVX mice with and without DHT injection, and the female controls. #P<0.05 vs. OVX mice.

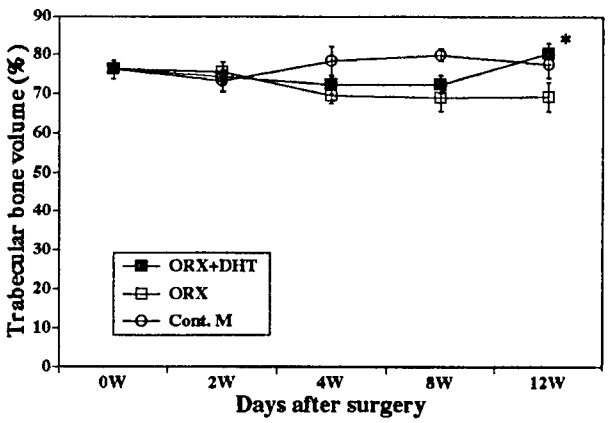

Figure 16. Changes in the trabecular bone volume in the ORX mice with and without DHT injection, and the male controls. *P<0.05 vs. ORX mice.

Time differences in the increase of trabecular bone volume between E2 and DHT injected mice are confirmed on the histologie sections. E2 injection produced a recovery of trabecular bone from 4 to 12 weeks after surgery as shown in Figure 17. Meanwhile, DHT injection produced a recovery of trabecular bone only 12 weeks after surgery (Figure 18).

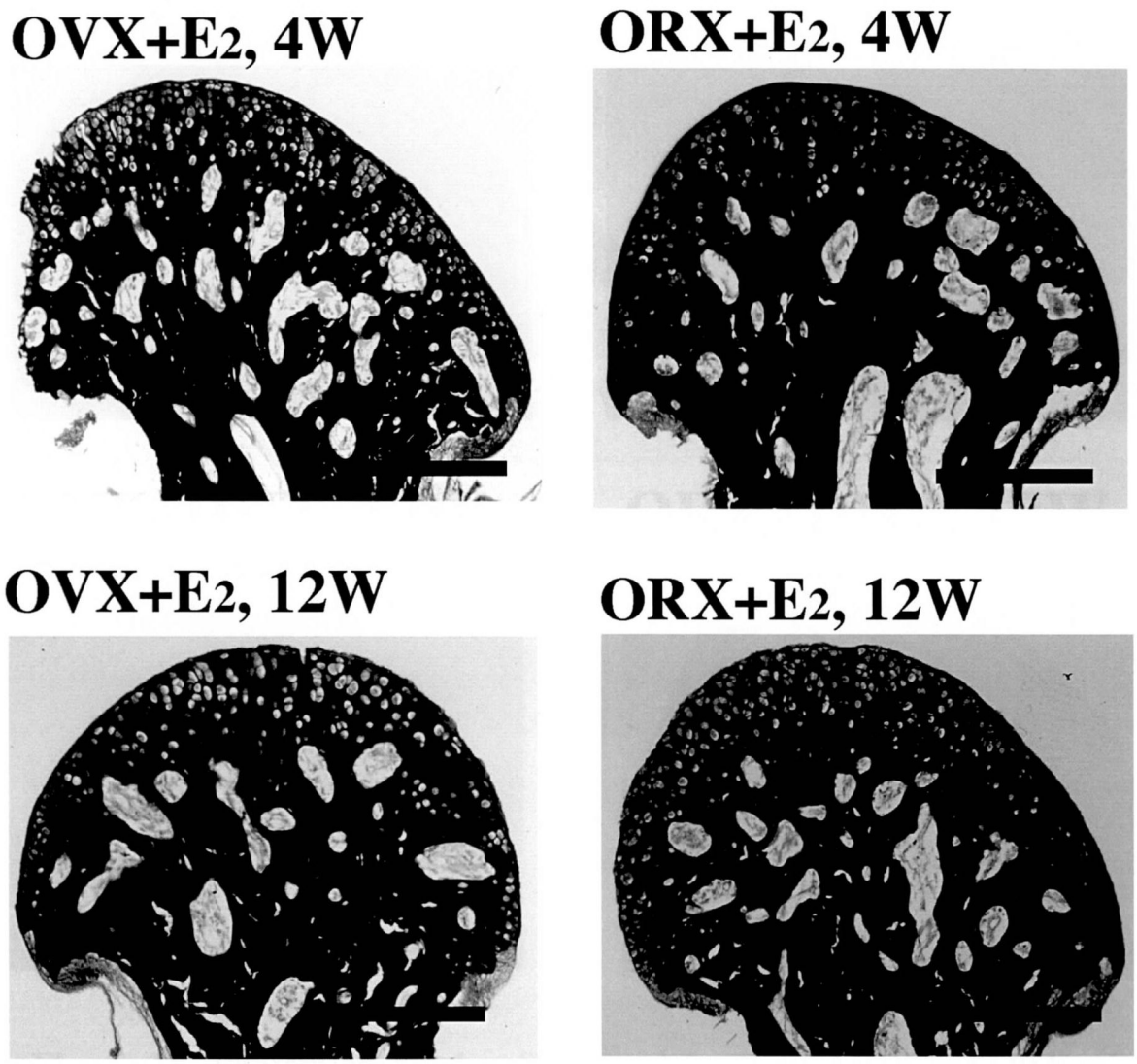

Figure 17. Photomicrographs of the condyles in the OVX and ORX mice with E2 injection 4 and 12 weeks after surgery. Bars denote 500 μm. (AZAN staining; $\times$100)

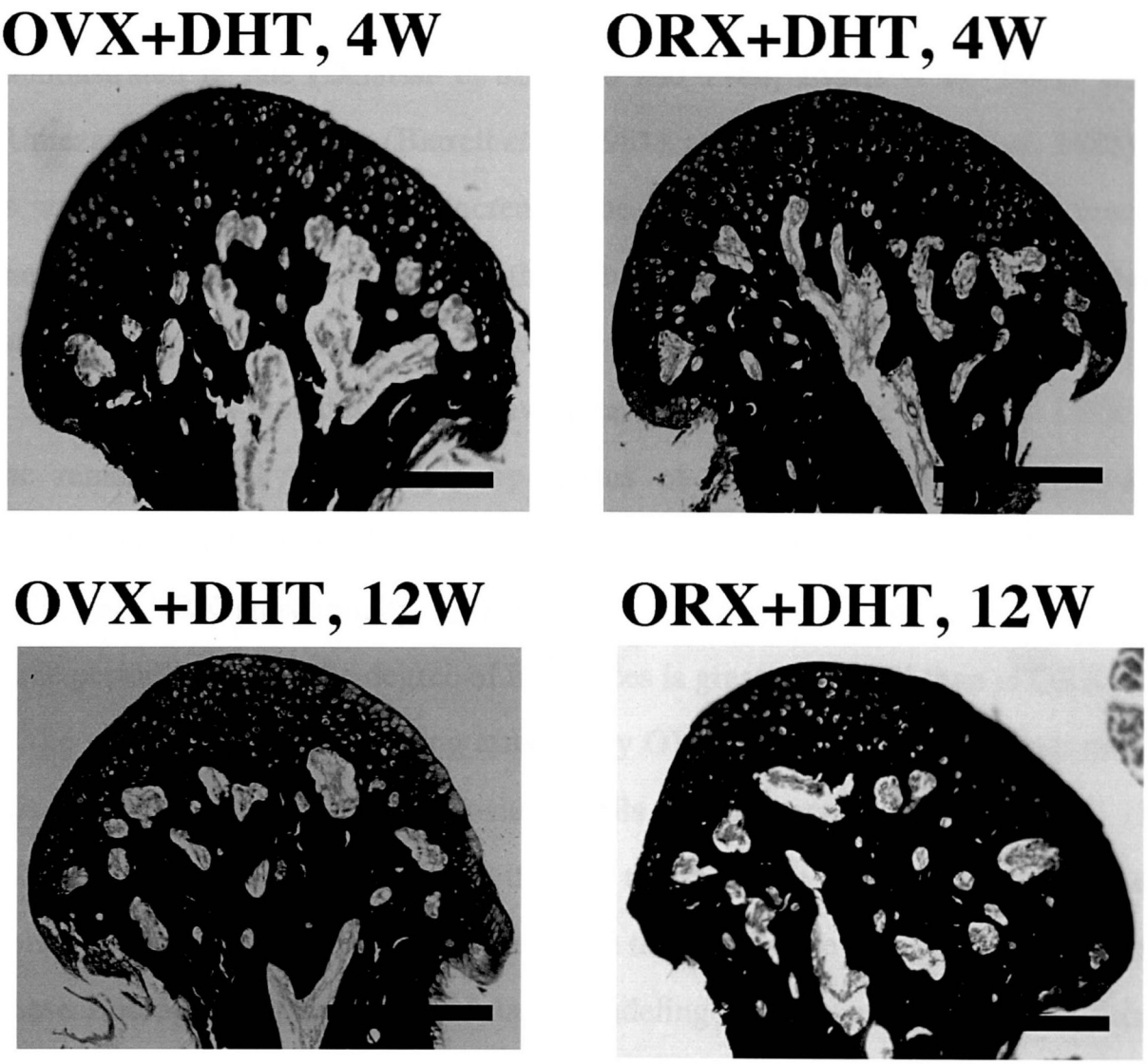

Figure 18. Photomicrographs of the condyles in the OVX and ORX mice with DHT injection 4 and 12 weeks after surgery. Bars denote 500 μm. (AZAN staining; $\times$100)

4. Discussion

It is currently accepted that OVX enhances the turnover of long bones such as the femora and tibia. The enhanced turnover is expressed as increased bone resorption and reduced new bone formation. Such phenomenon has already been demonstrated in rats (Delaisse *et al.* 1980 and 1984; Everts *et al.* 1985), dogs (Umezawa 1976), baboons (Barrett *et al.* 1981), and humans (Baron *et al.* 1985). It is well known that ORX also increases the bone turnover, however, the precise mechanism is still unclear. Further, the influences on condylar remodeling and the subsequent growth have not been studied.

This study was thus designed to investigate the influences of OVX and ORX on the remodeling of the condyle by means of histological, histochemical and histomorphometric methods. It has been demonstrated from the present study that OVX and ORX substantially influence the remodeling of condyle at almost the same period, although the degree of influences is greater in OVX than in ORX.

The nature of bone remodeling affected by OVX and ORX was demonstrated in this study in terms of TRAP-positive cells and trabecular bone volume. The increase rate of TRAP-positive cells and the decrease rate of trabecular bone volume in the condylar head were greater in the OVX mice than in the ORX mice. These findings suggest that condylar remodeling and the essential cartilaginous growth may be more substantially influenced by the sex hormone in female than in male, emphasizing that the timing and the mechanisms of the influences of estrogen and androgen on the expression of osteoclasts are different. Since androgens are converted into estrogens by skeletal aromatases (Tanaka *et al.* 1993), the effects of androgens may depend upon their conversion into estrogens. In order to study the effects of estrogen and androgen on bone resorption,

therefore, the effects of nonaromatizable androgens should be examined in comparison to these of estrogens.

In this study, the influences of E2 and DHT, injected into the OVX and ORX mice, on condylar remodeling have also been examined. The dosage of DHT was defined in this experiment as 10 times larger than that of E2, according to previous experiments (Gallagher *et al.* 1996; Sato *et al.* 1993). After the menopausal in women, the estrogen is produced by the function of aromatase in the fatty tissues, therefore, it may be assumed that the amount of estrogen is highly pertinent to the body weight. Since no remarkable difference in the body weights were found between two groups treated with E2 and DHT and the number of TRAP-positive cells was similarly suppressed in both groups, it may be confirmed that the dosages of E2 and DHT used in this experiment are quite reasonable.

The present study demonstrated that the increase in TRAP-positive cells and the decrease in trabecular bone volume observed in the OVX and ORX mice were obviously suppressed by the injection of E2 and DHT. It is well known that E2 suppresses osteoclasts differentiation mediated by IL-1 (Kimble *et al.* 1994), IL-6 (Jilka *et al.* 1992) and other cytokines, or directly through E2 receptor (Oursler *et al.* 1994). Meanwhile, DHT inhibits the differentiation of osteoclasts directly by decreasing IL-6 production with bone marrow cells (Bellido *et al.* 1995), and by inhibiting prostaglandin E2 production in tissue culture (Pilbeam and Raisz 1990), and osteoclastogenesis (Bellido *et al.* 1995), or directly through DHT receptors (Mizuno *et al.* 1994). However, it is unclear whether E2 and DHT suppress the enhancement in the number of osteoclasts on the condylar head of OVX and ORX mice. In this study, we examined the differences of influences on the condylar head between E2 and DHT injected into the OVX and ORX mice. The present

results indicate that E2 and DHT decrease the number of osteoclasts in the condylar head of OVX and ORX mice in an almost similar manner.

The trabecular bone volume in the OVX and ORX mice treated with DHT increased only 12 weeks after surgery, whereas E2 increased the volume from 4 to 12 weeks after surgery. Thus, the increase in the trabecular bone volume appeared earlier in the E2 injection group than in the mice treated with DHT. It has already been demonstrated that treatment with estrogen prevents bone resorption (Christiansen *et al.* 1982; Stock *et al.* 1985; Turner *et al.* 1987) and accelerates bone formation. Such effects of estrogen are regarded as the suppression of osteoclast differentiation (Girasole *et al.* 1992; Jilka *et al.* 1992; Kimble *et al.* 1994) and the promotion of mRNA expression in IGF-I (Ernst *et al.* 1989; Ernst and Rodan 1991) and TGF-ß (Komm *et al.* 1988). The function against bone resorption has recently been demonstrated as a direct effect of estrogen on bone metabolism (Pilbeam *et al.* 1989; Takano-Yamamoto and Rodan 1990), in which estrogen induces osteoblastic lineage cells to inhibit osteoclastic bone resorption (Tobias and Chambers 1991). Furthermore, an evidence of estrogen (Eriksen *et al.* 1988) and androgen (Colvard *et al.* 1989) receptors in osteoblasts has been presented, suggesting that the sex hormones have direct effects on bone metabolism. With respect to the mechanism of DHT, it has been confirmed in most studies that DHT stimulates proliferation of osteoblasts and osteoblast-like cells (Gray *et al.* 1992; Kasperk *et al.* 1989; Masuyama *et al.* 1992; Weisman *et al.* 1993) but not indicated elsewhere (Benz *et al.* 1991). DHT also stimulates differentiation of osteoblasts, as was demonstrated by an increased secretion of collagenous proteins or increased activities of such enzyme as creatine kinase, although DHT effects on alkaline phosphatase were not consistent (Benz *et al.*

1991; Gray *et al.* 1992; Kasperk *et al.* 1989; Weisman *et al.* 1993). However, the effects of DHT on osteoblasts in a molecular biology aspect almost remain unknown. It is demonstrated in this study that both E2 and DHT increase trabecular bone volume, although the difference of the influences on trabecular bone volume between E2 and DHT is unclear. These results suggest that the effects on the increase of trabecular bone volume between E2 and DHT may be different; *i.e.* E2 may promote the increase of trabecular bone volume more than DHT on the condylar head of the OVX and ORX mice.

In a clinical aspect, the decrease of trabecular bone volume common to hypogonadism was suppressed by the injection of testosterone. It is reported that aromatase transforms testosterone into E2 which further affects bone cells (Tanaka *et al.* 1993). Meanwhile, testosterone can also be transformed to DHT, therefore, it is currently beyond our knowledge to determine whether testosterone is transformed into either E2 or DHT. With these considerations, E2 was injected as estrogen and DHT was selected for androgen, because DHT is not transformed to E2 by aromatase. In living structures, an equilibrium between bone formation and resorption is well maintained through transmitting information for the status of bone remodeling. However, in this study, the trabecular bone volume was increased by the treatment with DHT only 12 weeks after surgery, although the number of osteoclasts decreased significantly during this period. Meanwhile, the OVX and ORX mice with E2 injection experienced the increases from 4 to 12 weeks after surgery. It may be assumed from these findings that testosterone is transformed mainly into E2 by aromatase, but all the testosterones are not altered into E2 in osteoblasts.

It is clinically recognized that the prevalence of TMDs, the osteoarthrosis with

condylar resorption in particular, is higher in female than in male. Therefore, it would be a reasonable assumption that prevalence of TMDs is related with sex hormones, estrogen of women in particular. Aufdemorte *et al.* (1986) and Milam *et al.* (1987) examined an existence of estrogen and progesterone receptors in primate TMJ. Female baboon TMJ has estrogen receptors, although the receptors were absent in the male. The presence of estrogen receptors in primate female TMJ suggests a potential relationship between estrogen-mediated cellular activities and preponderance higher susceptibility to TMJ problems in females. Recently, Abubaker and associates (1993) studied human TMJ for estrogen receptors with an immunohistochemical method. They found that 72% of symptomatic females and only 14% of asymptomatic females had immunodetectable estrogen receptors in the TMJ specimens. Finally, they derived such a feasible speculation that the concurrent presence of estrogen receptors and specific circulating hormone levels will lead to connective tissue alteration in the TMJ disk, causing some structural changes in the TMJ. These findings are consistent with a previous report (Tsai *et al.* 1992), which demonstrated an increase in estrogen and estrogen receptor density in osteoarthritic knee joints relative to normal joints. It is suggested, therefore, that symptomatic females may have different responses to estrogen levels based on available target receptors in the TMJ, although the exact role of estrogen in the pathogenesis of TMDs is unclear.

Furthermore, it is speculated that estrogen deficiency may induce an osteoporotic change in the subchondral bone, as is often observed in patients with TMDs (Carlsson and Öberg 1979; Toller 1973). It is suggested from the present results that estrogen strongly influences bone metabolism in the mandibular condyle of both men and women, therefore, it is assumed that higher prevalence of TMDs in

female may be related with the imbalance of hormone levels in women, which is frequently experienced as the changes of estrogen levels in puberty, menstruation, pregnancy and menopause.

5. Conclusions

The present study was conducted to examine histological and histochemical changes of the condyle in ovariectomized (OVX) and orchiectomized (ORX) mice and the differences between OVX and ORX mice subjected to the injection of estrogen (E2, 17ß-estradiol) and non-aromatizable androgen (DHT, 5∂-dihydrotestosterone). One hundred and seventy C57BL/6J mice of 8-week-old were used in this experiment. These mice were divided equally into six experimental groups with OVX, ORX, OVX+E2, ORX+E2, OVX+DHT, ORX+DHT, and non-treatment male and female control groups. In each experimental group, five mice were sacrificed 2, 4, 8 and 12 weeks after surgery for OVX and ORX. Estrogen and androgen were given daily after the surgery by subcutaneous injection of E2 and DHT. The following results were obtained.

1. Injection of E2 and DHT produced a remarkable increase in the body weight in comparison with the OVX and ORX mice without the injection. No remarkable differences in the body weights were found between the groups with E2 and DHT injection.

2. Increases in the number of TRAP-positive cells, induced in the OVX and ORX mice from 4 to 12 weeks after surgery, were obviously suppressed by the injection of E2 and DHT.

3. The trabecular bone volume in the OVX and ORX mice treated with DHT increased only 12 weeks after surgery, whereas E2 injection produced a substantial increase from 4 to 12 weeks after surgery.

It is shown that OVX and ORX substantially affect the remodeling of condyle. It is also elucidated that E2 injection into the OVX and ORX mice increases the trabecular bone volume earlier than the DHT, and that E2 and DHT suppress the

osteoclast differentiation similarly during the same period. These results suggest that metabolic responses of osteoclasts and osteoblasts to E2 and DHT must be varied, due to different patterns of bone remodeling in male and female.

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