

# THE EFFECTS OF OSTEOPOROSIS ON DISTRACTION OSTEOGENESIS : AN EXPERIMENTAL STUDY IN AN OVARIECTOMISED RABBIT MODEL

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The effects of oestrogen deficiency-associated osteoporosis on callus distraction were investigated in rabbits. Twenty-four female New Zealand rabbits 5 to 6 months old were used. Ovariectomy was performed on 12 rabbits, which composed the osteoporotic model group. Six weeks later, osteotomy was carried out and Ilizarov external fixators were applied to the right proximal tibial metaphyses in both the osteoporotic model group and the control group. Beginning one week postosteotomy, the metaphyses were distracted 0.35 mm twice daily for 3 weeks, and the average length increase obtained for both groups was 17.2 mm (minimum : 16.8, maximum : 19 mm). Following a postdistraction waiting period of 6 weeks for newbone formation, the subjects were sacrificed and specimens were examined histopathologically. Radiography was carried out at one-week intervals during the distraction period and at 2-week intervals during the waiting period, and scintigraphy was performed at the end of each period.

On histopathologic examination, a significant difference in callus remodeling was observed between the control and osteoporotic model groups. On radiologic evaluation it was observed that, while both groups had inadequate callus tissue at the end of the waiting period, callus formation and remodeling occurred later in the model group than in the control group, and the new bone was more osteoporotic.

Osteoporosis associated with estrogen deficiency adversely affects the outcome of callus distraction. Nonetheless, radiographic findings in rabbits indicate that the effects may not be so great as to preclude clinical procedures. It was concluded that these results should be supported with clinical studies.

## INTRODUCTION

Bone loss occurs throughout the early phase of oestrogen deficiency, and is concomitant with increased bone turnover. This phenomenon has been demonstrated in a number of biochemical and radiographic studies in rats, monkeys, and humans (2, 4, 8, 12, 13). In a histomorphometric study, Wronsky *et al* (11) observed trabecular bone loss in the proximal tibial metaphysis in ovariectomised rats, and determined it to be concomitant with increased longitudinal bone growth and accelerated bone resorption and formation.

Studies have been done on the changes produced by oestrogen in bone metabolism and the effects of these changes on fracture union. In biometric studies on tibial fractures in rats, Blythe and Buchsbaum (1) and Langeland (6) found no significant difference in tensile strength between ovariectomised and normal rats. Kubo *et al* (5), in osteoporotic animal models created by ovariectomy and a low-calcium diet, found osteoporotic changes and decreased bone-mineral density in new bone, and interpreted their

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findings to mean that oestrogen deficiency did not significantly affect the early phase of fracture union, but that it adversely affected the late phase.

The use of callus distraction in orthopaedic surgery has expanded in the past two decades, especially in the treatment of bone defects resulting from tumors, infection, and trauma. Bone defects are also encountered in osteoporotic patients. Although there is no clear upper age limit for callus distraction, its use is generally avoided in older patients because of increased complications. Although there are a number of studies on the effects of osteoporosis associated with menopause, natural or otherwise, on bone union (3, 5, 6, 7, 9, 10), its effects on callus distraction are relatively unknown. The purpose of this study was to investigate the effects of oestrogen deficiency on callus distraction and any complications that may arise.

## MATERIALS AND METHODS

Nonpregnant female New Zealand rabbits bred in the Animal Research Laboratory, Health Sciences Institute, Dicle University (DUSAM), weighing 1750-2500g and aged 5-6 months, were chosen as subjects, and the treatment of the animals was approved by the Ethics Committee of the Dicle University Medical Faculty.

A total of 24 rabbits was divided into 2 equal groups: the osteoporotic model group (group 1) and the control group (group 2). They were sheltered freely in individual cages, 40 × 45 × 35 cm.

### Osteoporotic rabbit model

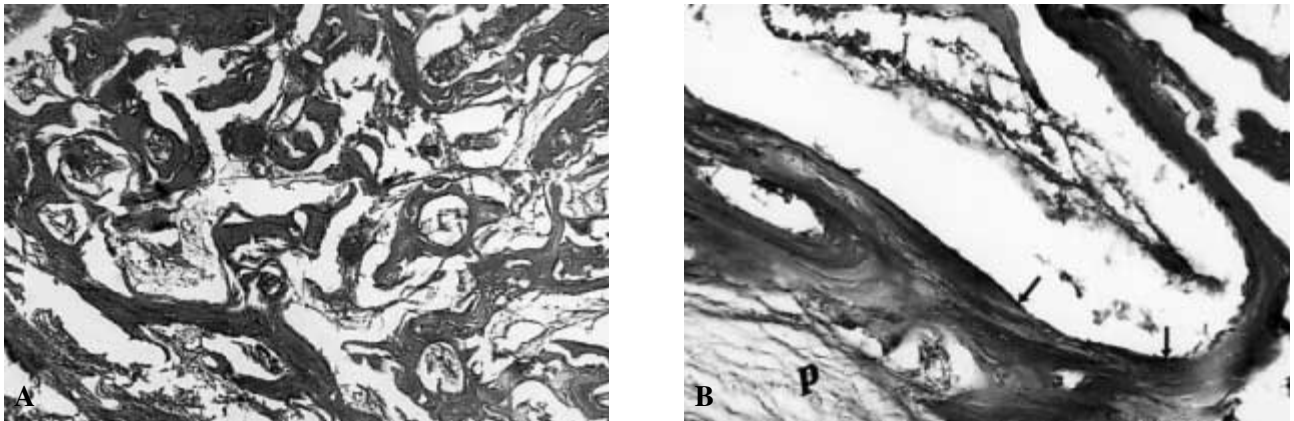
To the 12 rabbits in the osteopenic model group, 50 mg/kg ketamine was given intramuscularly for anesthesia, after which bilateral ovariectomy was performed. Ovariectomised rabbits were given 2 daily doses of 50 mg/kg ceftriaxone for 4 days for prophylaxis. Six weeks after ovariectomy, the study and control groups were given general anesthesia with the same dose of ketamine-HCL. A device similar to the Ilizarov circular external fixator was developed for use in rabbits. Four Kirchner wires 1.5 mm in thickness were applied, 2 to the right proximal tibial metaphysis and 2 to the distal metaphysis, and they were distracted. Two aluminum rings with an inner diameter of 45 mm, an outer diameter of 75 mm, a thickness of 4 mm, and a weight of 82 g were fastened with two stainless steel rods to achieve a

0.7 mm increase in length with one rotation of the nuts. Fibular osteotomy was performed in the distal diaphyseal region, and then tibial osteotomy was carried out in the proximal metaphyseal-diaphyseal region. After a waiting period of one week, radiographs and scintigrams were taken in both groups, and a 20-day distraction regimen of 2 × 0.35 mm/day was initiated. Afterwards, radiographs and scintigrams were taken again and, during a waiting period of 6 weeks for consolidation, radiography was repeated at 2-week intervals. At the end of the consolidation period, radiography and scintigraphy were again performed, rabbits were sacrificed with a lethal dose of Pentothal (75 mg/kg in the ear vein), and the distraction region was submitted to histopathologic examination.

**Radiological evaluation :** Distraction was begun in the osteoporotic model and control groups one week after osteotomy, and radiography (distance : 70 cm, voltage : 42 kVp, electric current : 6.4 mamp, time : 0.075 sc) was performed in the second week and the end of distraction, and in the second, fourth, and sixth weeks of the waiting period. The radiographs were evaluated by an orthopaedist blinded to the group composition.

**Histopathologic evaluation :** The callus distraction region of the tibia in the study and control groups was fixed with 10% neutral formalin for 24 hours. Afterwards, tissue samples were washed in running water and then left for 10 days in decalcification solution (solution A : 50 g sodium citrate, 250 ml distilled water ; solution B : 125 ml 90% formic acid and 125 ml distilled water mixed equally). Following decalcification, tissues were dehydrated in gradually increasing alcohol series (13). Following a paraffin bath, tissue samples were embedded in paraffin blocks so that longitudinal sections could be taken. Sections 3-4 mm in thickness were obtained with a rotary microtome, stained with hematoxylin-eosin (HE), and examined under the light microscope. Microphotographs were taken on an Olympus BH2 triocular microscope.

**Scintigraphic evaluation :** Scintigraphic images were obtained with a large field-of-view gamma camera (GCA-601, Toshiba, Japan) equipped with a low-energy, parallel-hole collimator (140 keV peak, 20% energy window). The animals were immobilised during image acquisition using diazepam. One mCi (37 mBq) technetium 99m-labeled methylene diphosphonate (MDP) (Mallinckrodt, The Netherlands) was injected intravenously into the ear vein. Serial images were acquired over the target leg and nontarget leg every second for 1 minute. A blood-pool image over the same area was obtained at 5 minutes with 5,000,000 counts. A bone-



**Fig. 1A.** — Appearance of the tibia in the control group ; note the regular, dense structure of the spongy trabeculae, and the red medulla (HE  $\times 100$ , original magnification). **B.** Section of callus tissue in the control group ; note endosteal cell covering (arrow), mature bone tissue, and periosteal (p) tissue (HE  $\times 200$ , original magnification).

phase image was obtained at 3 hours. Both the distraction area and the contralateral normal leg were included in the field of view. All data were transmitted to the computer for reporting. The images were analysed both visually and semiquantitatively.

## RESULTS

A total of 3 rabbits died, one in the osteoporotic model group one day after ovariectomy, and one in each group during callus distraction. In addition, cage injuries were determined on the legs on the callus distraction side in 3 rabbits ; these animals were not excluded from the study.

**Histopathological findings :** Tissue samples obtained 9 weeks after osteotomy in both groups were examined in a blind fashion.

Dense, regular structure was observed in spongy trabeculae of new bone taken from callus distraction regions in the control group (fig 1a). In the same group, it was observed that callus tissue stained like mature bone, and stained acidophilic with routine histologic stains, and that the lamellar structure was regular in the compact bone region (fig 1b).

Thin, irregular structures were observed in the callus tissues of ovariectomised rabbits (fig 2a). We observed that the callus in the periosteal region was not compact as it was in the control group, that the

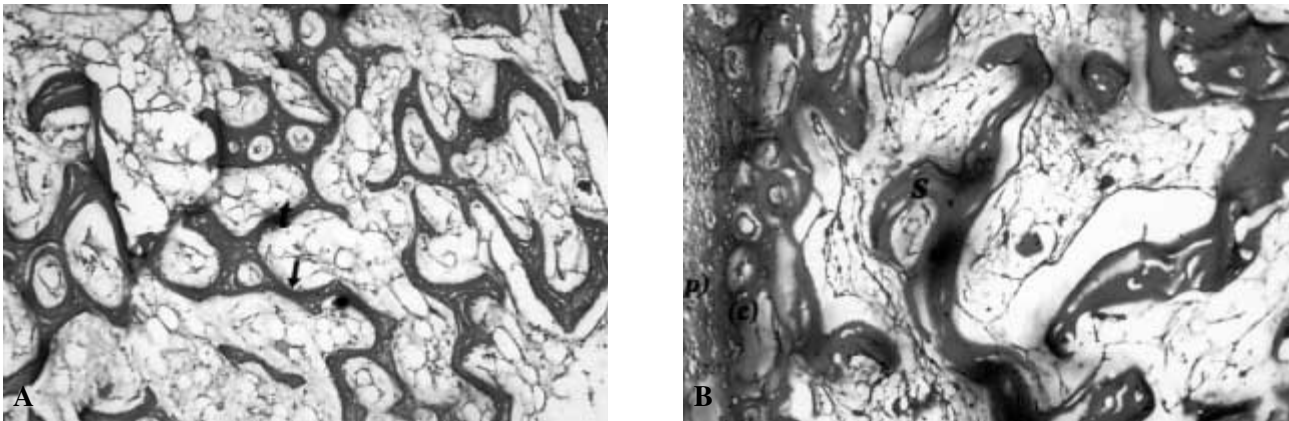
calcification of the matrix was inhibited, and that the bone structure was immature (fig 2b).

On histologic examination, a significant difference in callus remodeling was observed between the two groups.

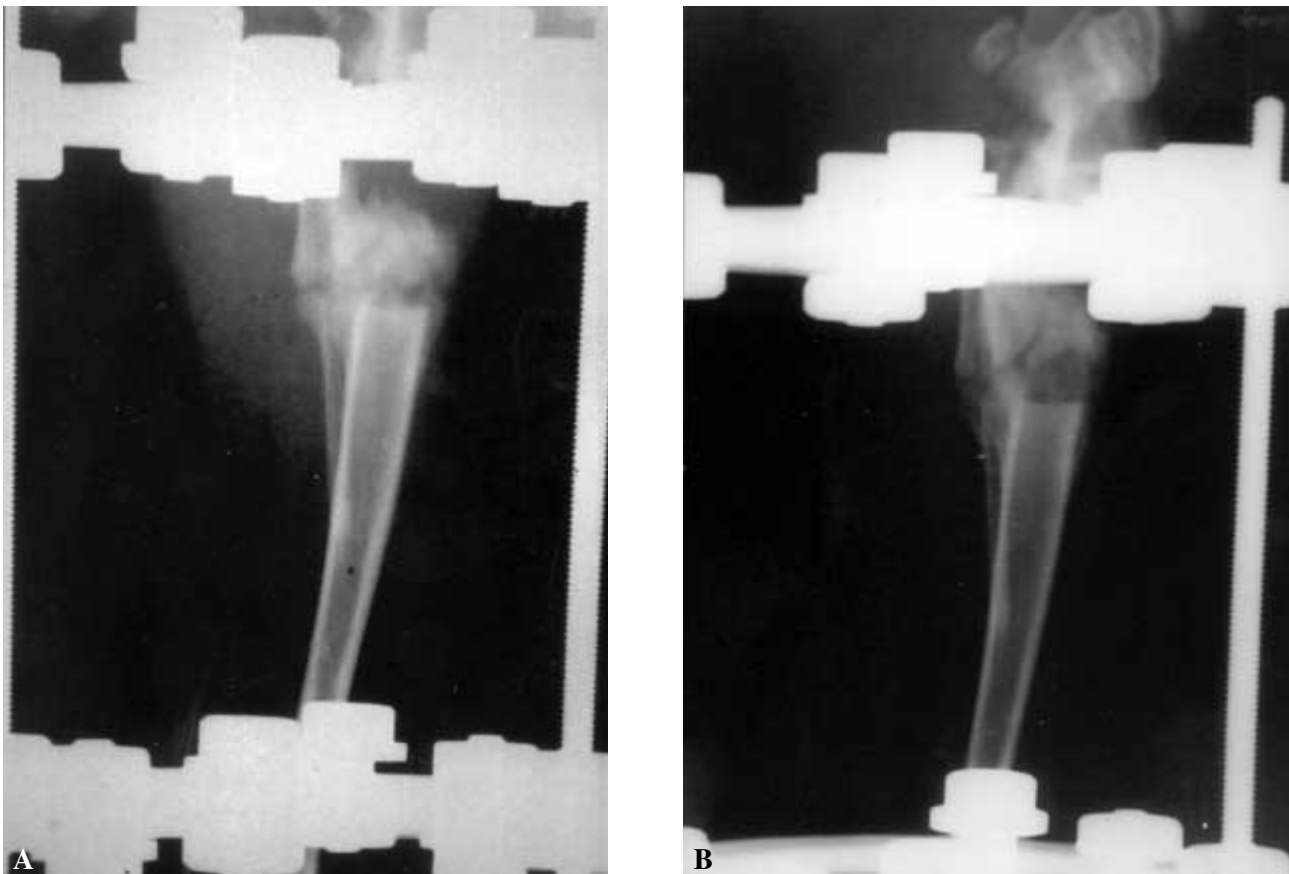
**Radiographic findings :** At the end of distraction, the average length increase obtained for both groups was 17.2 mm (minimum 16.8, maximum 19 mm).

**Control group :** On radiographs taken at the beginning and in the first week of distraction, no callus tissue was observed. On radiographs taken in the second week of distraction, slight callus tissue had begun to appear in the distraction zone. At the end of distraction, on radiographs taken in the third week, the callus tissue in the center of the zone was more distinct, and callus tissue had formed near the fractured bone segments. In the second week of the waiting period, callus tissue bridging from cortex to cortex was observed in three of the subjects. In the fourth week of the waiting period, bridging with a higher density of callus tissue was observed in all subjects in this group. However, there were radiolucent regions near the fragments' medullae, and there was less callus tissue (fig 3a). At the end of the waiting period, the density of the callus tissue was near that of normal bone, particularly in the center of the distraction zone (fig 3b).

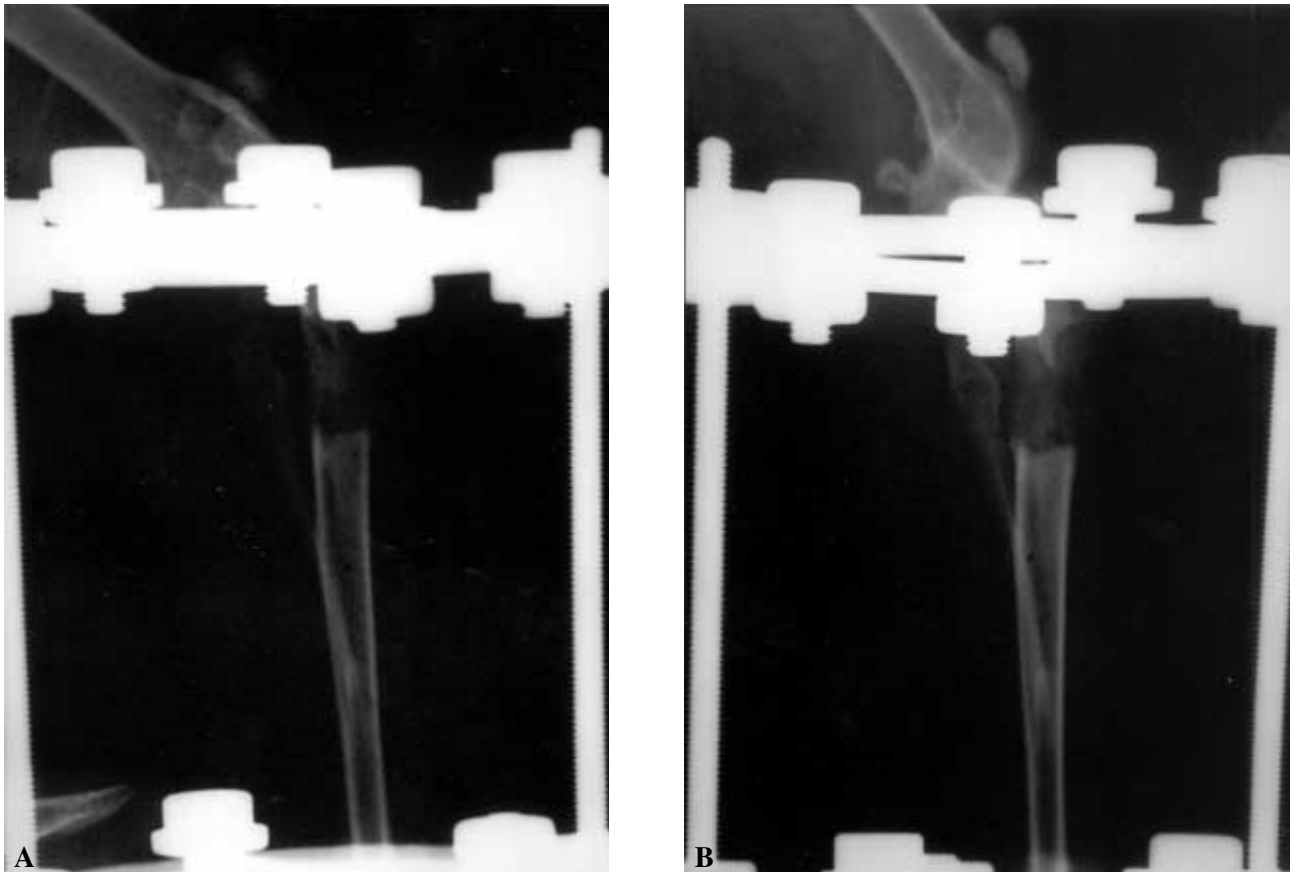
**Osteoporotic model group :** At the beginning and in the first week of distraction, no callus tissue



**Fig. 2A.** — Callus tissue in the osteoporotic model group ; appearance of endosteal cells (arrow). Note the irregular and very fine structure of the spongy trabeculae (t). **B.** Appearance of callus tissue in the osteoporotic model group ; note the periosteal region (P), the immature appearance of the new callus tissue (C), and the irregular and fine structure of the spongy trabeculae (S) (HE  $\times 200$ , original magnification).



**Fig. 3A.** — Radiograph from week 4 of the waiting period of a rabbit from the control group. In addition to the radiolucent regions in the fractured bone segments, note the callus bridging from cortex to cortex, and very dense callus tissue in the central zone. **B.** Radiograph of the same subject at the end of the waiting period. The radiolucent region at the fracture ends has disappeared, and the density of the callus tissue has reached that of normal bone.



**Fig. 4A.** — Radiograph of a rabbit from the osteoporotic model group at week 4 of the waiting period. Note that there is very little callus tissue and that there is low callus density in the central zone. **B.** Radiograph taken at the end of the waiting period. Despite callus bridging from cortex to cortex, there are still radiolucent regions near the bone ends, and the callus tissue has low density.

was observed. In the second week, slight callus tissue formation was visible in the center of the distraction zone in only four subjects. In the third week, while callus tissue was observed in the central section, none had yet formed near the bone segment end; during the same period, callus formation had also begun near the fractured bone segments, but there was no osseous bridge in any of the subjects, and there were transverse radiolucent regions between the fragments and the distraction zone. In the fourth week of the waiting period, callus bridging had occurred in all but 3 subjects (fig 4a). On radiographs taken at the end of the waiting period, callus bridging had occurred between fragments in all subjects, although with lower density (fig 4b).

At the end of the waiting period, although callus tissue was inadequate in both groups, the formation

and remodeling of callus tissue occurred later, and the new bone had a more osteoporotic appearance in the osteoporotic model group than in the control group.

**Scintigraphic findings:** Comparisons were made between scintigraphs taken in the first and last (third) weeks of distraction; between scintigraphs taken in the blood pooling phase and the late phase; between scintigraphs taken in the osteotomy region and the symmetric region of the contralateral leg; and between scintigraphs taken on the osteotomy side and symmetric side in the control group and in the study group.

In the first week of distraction in the control and osteoporotic groups, there were significant differences between the osteotomy side and the contralateral side in counts made in both the blood

pooling phase and the late phase, with more activity on the osteotomy side ( $p < 0.05$ ). Although activity on both sides was higher in the control group than in the study group, the difference was not significant ( $p > 0.05$ ).

In counts made from scintigraphs taken at the end of distraction (week 3) in both groups, the increase in activity on the osteotomy side was greater than in the contralateral extremity in both phases ( $p < 0.01$ ) and was visually observable. Although the increased activity on the osteotomy sides was not significantly different between groups in counts made in the blood pooling phase, activity was significantly higher in the osteoporotic model group in the late phase ( $p < 0.05$ ), but no significant difference was found between contralateral extremities ( $p > 0.05$ ).

## DISCUSSION

According to Ilizarov's traction-stress theory, which is the basis of distraction osteogenesis, continuous traction creates stresses that stimulate active growth in many living tissues. As a result of slow continuous traction, tissues become metabolically active, and proliferation and biosynthesis increase. The primary reason for this increase is increased blood flow to the tissue. The effects of oestrogen deficiency on distraction osteogenesis and on the resulting new bone structure are not well known.

In the literature, there are differing opinions on the effects of oestrogen deficiency on fracture union and on the soundness of new bone. A number of biomechanical and histological studies indicate that it is significantly compromised, while very few report the opposite (1, 2, 9, 10, 11). There are two points of view on the mechanism of the effect of estrogen deficiency on bone union and new bone structure. Cesnčaj *et al* (2) investigated the osteoinductive effects of oestrogen and determined that osteogenesis was decreased and chondrogenesis retarded in ovariectomised rats. The authors explained this finding by hypothesising that oestrogen deficiency altered the production of osteoinductive proteins such as osteogenin and bone mor-

phogenetic protein, thus disturbing bone matrix formation. This mechanism accounts for the effect of oestrogen deficiency on the early phase of bone union. According to a different view, in addition to the negative effects on new bone formation, increased bone turnover will lead to osteoporosis in the new bone as well as in the host bone, and thus the new bone will be less sound. Kubo *et al* (5) assert that this mechanism adversely affects the late phase of bone union. Walsh *et al* (9) have determined biomechanically that new bone is less sound in ovariectomized rats, but that, in contrast with Kubo *et al's* findings, bone structure is less affected in the late phase, approaching that of the control group. In the present study, we observed in the study group a less compact structure in the callus formation in the periosteal region, inhibition of matrix calcification, and a less mature trabecular appearance. These findings suggest that oestrogen deficiency affects callus distraction as it does bone union.

Histomorphometric analysis of osteoporosis associated with menopause, a natural cause of oestrogen deficiency, has shown that bone resorption and formation are relatively normal in most osteoporotic patients (8, 12). However, there are subgroups of patients in whom bone resorption is high and formation is low. These heterogeneous findings are explained by the hypothesis that there may be different phases in osteoporosis associated with oestrogen deficiency and that osteoblastic and osteoclastic activity may vary from phase to phase. Higher increases in osteoblastic and osteoclastic activity in the early phases of oestrogen deficiency have been reported. In our study, the fact that the increase in activity in the late metabolic phase was higher in the osteoporotic model group than in the control group on scintigrams taken during week 3 supports the view that both activities are increased in the early phase of oestrogen deficiency.

Radiographic evaluation showed that, in the model group, callus tissue appeared later, callus bridging occurred with a delay of 1 to 2 weeks, and the callus tissue was more osteoporotic; nonetheless, there were no cases of nonunion or inadequate union in any of the subjects. We think that these findings are clinically significant.

According to histopathologic and radiologic findings in this study, when callus distraction is carried out in subjects with estrogen deficiency, new bone forms more slowly and is more osteoporotic. Nonetheless, the fact that callus formation was sufficient, albeit delayed, in all subjects in the model group indicates that callus distraction may be used clinically, even in oestrogen-deficient patients. However, a longer healing index and a greater risk of refracture after fixator removal are to be expected due to osteoporosis. In clinical use, the delay in new bone formation and remodeling can be compensated for by extending the postosteotomy waiting period and by slowing distraction to a certain extent.

### CONCLUSION

Although osteoporosis associated with oestrogen deficiency has adverse effects on callus distraction, radiographic findings in rabbits show that these are not so great as to preclude clinical procedures. It was concluded that these results need to be supported by clinical study and, also, further experimental investigation of oestrogen administration in the osteoporotic model to see whether normal bone healing will be obtained, as this could be of benefit in clinical conditions. In callus distraction carried out in patients with oestrogen deficiency, of either natural or artificial etiology, a longer healing index and increased complications associated with osteoporotic bone are to be expected.

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