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Review Article

Hylonome

# Overview: animal models of osteopenia and osteoporosis

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**Abstract**

Prior to initiating a clinical trial in a post-menopausal osteoporosis study, it is reasonable to recommence the evaluation of treatment in the 9-month-old ovariectomized female rat. A female rat of this age has reached peak bone mass and can be manipulated to simulate clinical findings of post-menopausal osteoporosis. Ample time exists for experimental protocols that either prevent estrogen depletion osteopenia or restore bone loss after estrogen depletion. More time can be saved by acceleration of the development of the osteopenia by combining ovariectomized (OVX) plus immobilization (IM) models. Methods like serum biochemistry, histomorphometry and densitometry used in humans are applicable in rats. Like most animal models of osteopenia, the rat develops no fragility fractures, but mechanical testing of rat bones substitutes as a predictor of bone fragility. Recent studies have shown that the prevailing activity in cancellous and cortical bone of the sampling sites in rats is remodeling. The problems of dealing with a growing skeleton, the site specificity of the OVX and IM models, the lack of trabecular and Haversian remodeling and the slow developing cortical bone loss have been and can be overcome by adding beginning and pre-treatment controls and muscle mass measurements in all experimental designs, selecting cancellous bone sampling sites that are remodeling, concentrating the analysis of cortical bone loss to the peri-medullary bone and combining OVX and IM in a model to accelerate the development of both cancellous and cortical bone osteopenia. Not to be forgotten is the distal tibia site, an adult bone site with growth plate closure at 3 months and low trabecular bone turnover and architecture similar to human spongiosa. This site would be most challenging to the action of bone anabolic agents. Data about estrogen-deplete mice are encouraging, but the ovariectomized rat model suggests that developing an ovariectomized mouse model as an alternative is not urgent. Nevertheless, the mouse model has a place in drug development and skeletal research. In dealing with drug development, it could be a useful model because it is a much smaller animal requiring fewer drugs for screening. In skeletal research mice are useful in revealing genetic markers for peak bone mass and gene manipulations that affect bone mass, structure and strength. When the exciting mouse glucocorticoid-induced bone loss model of Weinstein and Manolagas is confirmed by others, it could be a significant breakthrough for that area of research. Lastly, we find that the information generated from skeletal studies of nonhuman primates has been most disappointing and recommend that these expensive skeletal studies be curtailed unless it is required by a regulatory agency for safety studies.

**Keywords:** Animal Model, Ovariectomy, Immobilization, Osteopenia, Osteoporosis

## Introduction

Osteoporosis is a disease characterized by a decrease in bone mass (osteopenia) and a deterioration in bone micro-architecture which leads to an enhanced fragility of the skeleton, and therefore to a greater risk of fracture. The study group of the World Health Organization (WHO) has qualified this definition as to state osteoporosis is present when the bone mineral density (BMD) or bone mineral content (BMC) is over 2.5 standard deviation (SD) below the young adult reference mean (-2.5 T-score). If fractures are present, the condition is known as "severe" osteoporosis. If one agrees with the decrease in BMD of 2.5 SD below the young adult reference with no fractures as osteoporosis[1], and not osteopenia[2,3], then currently there exist two well-established small animal models of local osteoporosis: the rat ovariectomy (OVX) and the immobilization (IM)-induced bone loss models.

The site-specific development of cancellous osteopenia in these models is one of the most certain biological responses in skeletal research. The following attributes of each model will be reviewed:

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1. Site specificity
2. Time course of the transient and steady state responses in cancellous and cortical bone loss as evaluated by static and dynamic histomorphometry
3. The biomechanical strength testing of select sites.

The shortcomings of these models will be detailed specifically concerning:

1. The need for beginning and pre-treatment controls
2. The need for muscle mass measurements
3. The site specific sampling problem for the OVX and IM models
4. The need for more study of adult bone sampling sites
5. The slow developing cortical bone loss
6. The lack of Haversian or intracortical remodeling associated with cortical osteopenia.

Suggestions will be given on how to overcome these shortcomings, to employ these models to further our understanding of the pathophysiology of osteoporosis and to meet regulatory demands in developing agents in the prevention and treatment of osteoporosis. In addition, the role of the OVX mouse and nonhuman primate and the mouse glucocorticoid-induced bone loss models as they contribute to osteoporosis research will be discussed.

## The rat skeleton

The Food and Drug Administration (FDA) guideline has appropriately designed the need for rat experimentation in the preclinical evaluation of agents used in the prevention or treatment of postmenopausal osteoporosis[4]. The ovariectomized rat is an excellent preclinical animal model that correctly emulates the important clinical feature of the estrogen depleted human skeleton and the response of therapeutic agents[5]. Its site-specific development of cancellous osteopenia/osteoporosis is one of the most reproducible biologic responses in skeletal research. The predominant cellular activity on endosteal (cancellous or trabecular and endocortical) bone surface is remodeling[6,7] contrary to the impression given in the FDA guidelines (Fig. 1). In addition, bone loss in aging occurs at endosteal surfaces adjacent to the marrow[8] (Fig. 2). Even the cortical bone displays a low level of intracortical remodeling in the rat that is readily induced by stressful metabolic conditions[9,10]. The major drawback of the rat skeleton is that some bones retain lifelong growth and do not fuse epiphyses[11]. Many long bone epiphyseal growth plates in the male rat remain open past 30 months[11]. In contrast, bone elongation at other sites like the proximal tibia and distal tibia ceases at 15 months and 3 months in a female rat[6,11-13], and the lumbar vertebral growth plates are open as late as 21 months (personal communications). A female rat at 9 months exhibits a slowed rate of elongation at the proximal tibia (PTM) of 3 μm/d, femoral head of $< 1\mu m/d$[14-17] and the distal tibia epiphyseal growth plate is closed[11,26] (Table 1). Periosteal expansion at long bone diaphysis continues until about 10 months, marking the age of peak bone mass[16-17] allowing ample time for experimental designs to prevent and restore bone mass and strength.

## The ovariectomized rat model

Following ovariectomy (OVX), rapid loss of cancellous bone mass and strength occurs, which then proceeds in a less rapid rate in a site-specific fashion to reach steady state phase of bone mass with an increase in rate of bone turnover (Fig. 3)[18-25]. These bone loss features mimic the bone changes

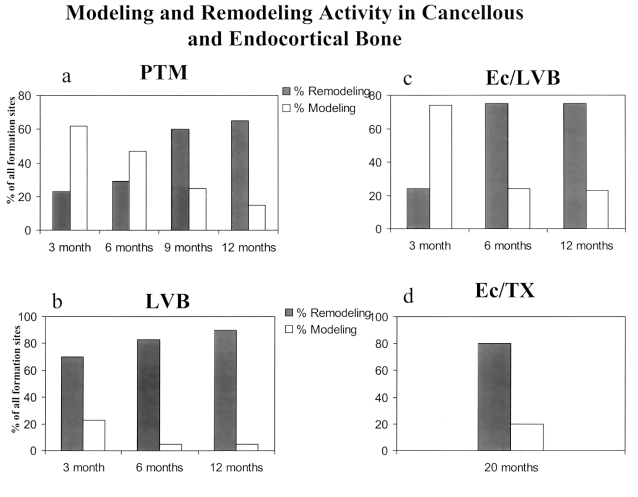


**Figure 1.** Modeling and remodeling activity in cancellous bone of the proximal tibial metaphysis (PTM, **a**), first lumbar vertebral body (LVB, **b**) and endocortical bone of the first lumbar vertebral body (Ec/LVB, **c**) of 3-12-month-old female Fisher rats and endocortical bone of the tibial shaft (Ec/TX, **d**) of 20-month-old male Wistar rat. Note the prevailing activity is that the older rat is remodeling at all 4 sites. Adapted from Erben[6] and Yao[7].

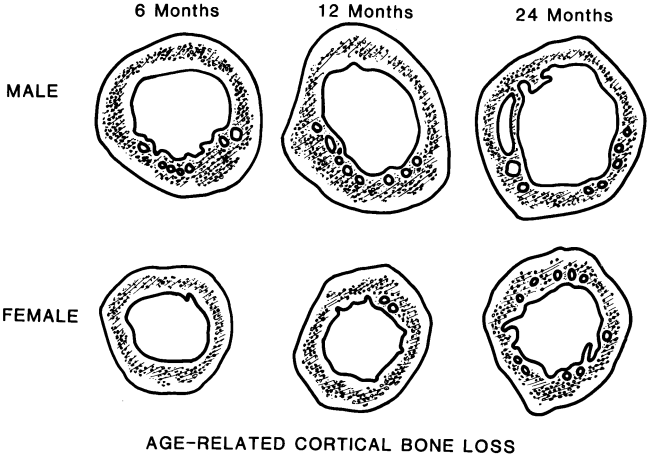


**Figure 2.** Diagram of mid femoral shaft in male (**top**) and female (**bottom**) Wistar rats at 6, 12, 24 months of age. Note the cavitation and the thinning of the cortex originating with the marrow cavity and the compensatory periosteal bone apposition. Both activities were much more apparent in the male rat. Adapted from Hagaman et al.[8]

| Site | LBG** at 9 months | Cancellous bone changes post-OVX in days (d) | | | | Refs. |
|---|---|---|---|---|---|---|
| | | Earliest time of bone loss | Time of 50% bone loss | Earliest to achieve steady state | Earliest decrease in bone strength | |
| PTM | 3 μm/d | ≈ 14d | ≈ 30-60d | 90d | -- | 18,19 |
| LVB | < 1 μm/d | ≈ 60d | ≈ 180-270d | 270d | 90d | 20(43,45) |
| FN | < 1 μm/d | ≈ 30d | ≈ 180-270d | 270d | 90d | 14(39,44) |
| DTM | closed | none | none | none | -- | 26 |

The times (d) listed may be less than that listed due to lack of short term studies; **LBG – longitudinal bone growth, PTM – proximal tibial metaphysis, LVB – lumbar vertebral body, FN – femoral neck, DTM – distal tibial metaphyses, -- no determination, ( ) bone strength references.

**Table 1.** Summary of cancellous bone changes post-ovariectomy.

following oophorectomy or menopause in humans. Not all cancellous bone sites in the rat exhibit such bone loss [26-27] nor do all cancellous bone sites lose bone at the same rate[14,18-20]. Table 1 shows the earliest statistically significant time of cancellous bone loss in the proximal tibial metaphysis (PTM), lumbar vertebral body (LVB) and femoral neck (FN) occurring at 14, 60 and 30 days, respectively, and slowing down at 90 days for the PTM[18,19] and 270 days for the LVB[20] and FN[14].

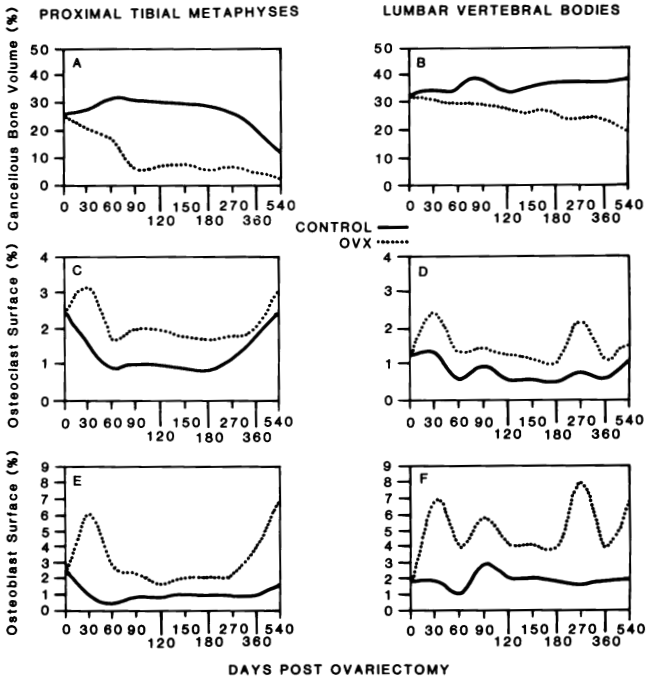


**Figure 3.** Time course changes in cancellous bone volume, osteoclast and osteoblasts surface in proximal tibial metaphyses (PTM,) and lumbar vertebral bodies (LVB,) of ovariectomized rats. There was much more bone loss in the PTM (**A**) than the LVB (**B**). The PTM arriving at steady state or plateau at 90 days versus LVB at 270 days. Both sites were at high turnover condition at steady state (**C-F**). Adapted from Wronski et al.[18-21]

In contrast, ovariectomy-induced bone loss does not occur in trabecular bone of long bone epiphyses, the distal tibial metaphysis and caudal vertebra[26,27,29,30].

In cortical bone of the mid-shaft or diaphysis of long bones, OVX stimulates periosteal bone growth[31,32]. On the other hand, the mid-diaphyseal endosteum in the OVX'd rat exhibits increased bone resorption leading to an enlargement in the size of the medullary cavity[31,33,35-42]. As a result of these combinative changes, cortical bone changes only slowly[22] so that the femoral shaft at 540 days post-OVX fails to demonstrate changes in BMC[42]. This is due to the fact that bone lost at the endosteum adjacent to marrow is being replaced on the adjacent periosteum. Since the most sensitive index of cortical bone loss involves the enlargement of the marrow cavity from the resorption of endocortical bone adjacent to marrow, a measurement of the thickness of the inner 1/2 or 1/3 of the cortex adjacent to the marrow proves to be meaningful. Danielsen and colleagues[35] reported a decrease in thickness of inner zone of femoral shaft (inner half of mid-diaphyseal cross section) at 3 months post ovariectomy. However, a decrease in bone strength was not apparent until 15 months post ovariectomy. Nevertheless there have been several studies indicating the earliest changes in cortical bone width and medullary cavity size to be between 90 - 120 days and to reach a steady state often 180 days or more (Table 2).

The rat, like other experimental animal models of osteopenia/osteoporosis, has no naturally occurring fragility fractures associated with the osteopenia. This shortcoming has been overcome by mechanical testing of various bones such as the vertebral body[43-48], femoral shaft[33,35,44,46,49,50], and proximal femur[34,39,44,46,50,51]. Tables 1 and 2 summarize the effects of ovariectomy on bone mass and biomechanical properties. Both significant loss of vertebral cancellous bone and strength can be detected by 3 months post ovariectomy[43,45,47]. The cortical bone of the femoral diaphysis showed an early transient increase in bone strength but decreased after 9 months[33,35,46,49]. In contrast, the cortical bone of the femoral

| Site | Earliest significant change in days post OVX* | | | | Refs. |
|---|---|---|---|---|---|
| | ↓Cortical width | ↑Medullary area | ↓Inner zone | ↓Bone strength | |
| TX | ≈ 180d | ≈ 90d | -- | -- | 40 |
| FX | ≈ 180d | ≈ 270d | ≈ 180d | 270d** | 33,35,47 |
| FN | ≈ 90d | ≈ 150d | -- | ≈ 63/90d | 34,39,47 |

TX - tibial shaft, FX - femoral mid-shaft, FN - femoral neck; ↓ decrease; ↑ increase
*Since none of these were time course studies, the times listed may all be less than the days listed. These rats were ovariectomized between 3 and 6 months of age. **Early transient increase in bone strength at 3 months but decreased after 9 months.

**Table 2.** Summary of cortical bone changes post-ovariectomy.

neck exhibited an earlier appearance in decreased bone strength at 3 months post ovariectomy[39,46,52].

## The immobilized rat model

Immobilization (IM) induced osteopenia/osteoporosis is another rat skeletal model with the highly predictable pattern of bone loss. Methods to reduce skeletal biomechanical loading include local or systemic immobilization[53,54]. The local immobilization or disuse model usually are performed in one limb. Other methods of disuse include nerve[55-60], spinal cord[56,61] or tendon resections[62-65], casting[57,66-70], bandaging of one limb[15,71-75] or suspension of both hindlimbs[76,77] in rats. The most frequently employed disuse models are tail suspension, nerve resec-tions, tendon resection and taping or casting of one limb in rats (Table 3). All of these models elicit similar skeletal responses with the predominant endpoint being site-specific bone loss. The different disuse models differ only in the speed of bone loss depending upon whether there is a regional acceleratory phenomenon (RAP) response from surgery. The RAP constitutes a considerable acceleration of all normal tissue turnover processes adjacent to an irritated intervention like surgery[78]. Because the RAP increases regional or local bone remodeling it typically is associated with increased bone loss next to marrow.

The classical immobilization-induced bone loss response can effectively be illustrated from the studies of unilateral one-hindlimb immobilization studies in rats and dogs[15,66-69,71-74]. The rate of bone loss in hindlimb immobilization is related to the level of normal bio-mechanical stress and strain to the bone. More bone loss in the immobilization model is seen in the weight-bearing lowers extremities than the non-weight-bearing upper extremities or in the case of tetrapods, the distal part of the limbs[67]. In addition, it has been shown that the cancellous bone in caudal vertebrae loses less bone than weight-bearing bones with immobilization[79]. Animals or bones with higher peak bone mass lose more bone than those with lower bone mass. Furthermore, trabecular bone loss occurs much faster than that in cortical bone after immobilization. This is due in part to the difference in surface to volume ratio and increased surfaces adjacent to marrow in trabecular-rich regions. The immobilization or unloading evokes a rapid, transient (acute) remodeling-

| Attributes | Suspension[76*] | Limb taping[71] | Nerve resection[58,65] | Tenotomy[62-64] | Limb casting[58,66**] |
|---|---|---|---|---|---|
| Site | Hindlimb | One hindlimb | One hindlimb | Lower hindlimb; foot | One limb |
| Surgery | No | No | Yes (sciatic or femoral nerve) | Yes (Knee calcaneal) | No |
| Hardware | Specialized cages; tail hardness | No (tape) | No | No | No (plaster cast) |
| Time frame | Short term < 5wks | Long term | Long term | Short term (tendon re-growth) | Long term |
| Responses | | | | | |
| Blood flow affected | Yes | Potential problem | Potential | ? | ↓(?) |
| Cellular fluid Shift | Yes | No | No | No | No |
| Muscle function | Yes | Restricted | No | Mildly affected | No |
| Nerve function | Yes | Yes | No | No | Yes |
| Cancellous bone loss | No | ↓(50) | ↓(50)(72)[58] | ↓(50) | ↓(60)[66,5*](68)[58] |
| Tb Formation | ↓(66) | ↓(35) | ↓(50) | ↓(45) | ↓ |
| Tb Resorption | No | ↑(50) | ↑(150) | ↑(125) | ↑ |
| Cortical bone loss | No | ↓(10) | ↓(4) | - | ↓(50)[66](14)[55] |
| Formation (Ps) | ↓(85) | ↓(90) | ↓(40) | - | ↓ |
| Resorption (Ec) | No | ↑(19) | ↑(100) | - | ↑ |
| Muscle weight | ↓(48) | ↓(55) | ↓(70) | - | ↓ |
| Convenience | Daily care | Daily care | Minor care | Excellent | Weekly care |
| Recovery possible | Yes | Yes | No | Possible; unpredictable | Yes |

*Findings only from older rats studies are cited; Ps-periosteal; Ec-endocortical; Tb, trabecular; ↑ Increase(%); ↓ decrease(%); - no data; cortical width.
**data from adult dogs.

**Table 3.** Summary of in vivo models for unloading (Immobilization) model of the rat skeleton*

| Site | Earliest cancellous bone changes post-IM in days (d)* | | Refs. |
|---|---|---|---|
| | Detectable bone loss | Time to achieve steady state | |
| Proximal tibial metaphysis | ≈ 14d | ≈ 126d | 71, 74 |
| Distal tibial metaphysis | ≈ 14d | ≈ 45d | 26, 72, 73 |
| Caudal vertebral body | ≈ 21d** | -- | 79 |

| | Earliest cortical bone changes post-IM in days (d)* | | | | |
|---|---|---|---|---|---|
| | ↓ bone | ↓ width | ↑ Med.Ar | ↓ bone strength | Refs. |
| Tibial shaft | ≈ 42d | ≈ 42d | ≈ 42d | ≈ 42d | 15, 75-80 |
| Femoral shaft | ≈ 21d | -- | ≈ 21d | > 84d | 75 |
| Femoral neck | -- | -- | -- | ≈ 21d | 81 |

*These times are estimates due to the lack of good time course data; -- not determined;
**only time studied, bone formation rate reduced by 12%

**Table 4.** Summary of bone changes post immobilization (IM).

dependent trabecular bone loss associated with an increase in resorption and a decrease in bone formation which reduces bone mass adjacent to marrow (Fig. 4)[15,71,74]. This is initiated in cancellous bone as early as 3 hours post tenotomy[63]. Other models of immobilization-related bone loss that avoid surgery and the RAP response occur at a slightly slower rate. At steady (chronic) state, the bone loss has plateaued with bone cellular activities back to control levels[15,71,74]. In the rat, the unilateral hindlimb immobiliza-tion (IM) model by bandaging, the earliest statistically significant cancellous and cortical bone loss was 14-30 days and 70 days, respectively[15,71,74]. The steady state occurred between 70 and 126 days in cancellous bone and more than 182 days post IM in cortical bone (Table 4). In the dog casted forelimb model, rapid cancellous bone loss slowed down at 126 days and cortical bone loss at more than 420 days[66]. All the rat hindlimb immobilization models resulted in about a 60% cancellous and less than 10% cortical bone loss (Table 3). Possibly there would be more cortical bone loss if the rat studies were carried out longer, as Jaworksi and Uhthoff[66] reported that the metacarpal in the dog lost up to 50% of its cortical bone after 60 weeks of forelimb casting (Fig. 4). Both transient elevated cancellous bone resorption (% eroded area, Fig. 5C) and depressed bone formation (Fig. 5E) combine to accelerate the cancellous bone loss (Fig. 5A). In contrast, in cortical bone there was an immediate near cessation of periosteal bone formation (modeling in the formation mode, Fig. 5F) and a significant increase in endocortical resorption (% endocortical eroded surface, Fig. 5D) that evoked the slow loss of compacta (Fig. 5B) and a non-significant enlargement of the marrow cavity at 182 days[15]. Laborious, longer term studies are needed to determine whether the rat will react like the dog in immobilization-induced cortical bone loss. Regardless, the important observations in cortical bone loss are the stimulation of endocortical bone resorption and the immediate depression in periosteal bone formation. There are profound architectural changes in the loss of trabecular connectivity and the conversion of trabecular plates to rods. In addition, decreases in mechanical properties occur as early as 21 days post-immobilization in the femoral shaft (Table 4).

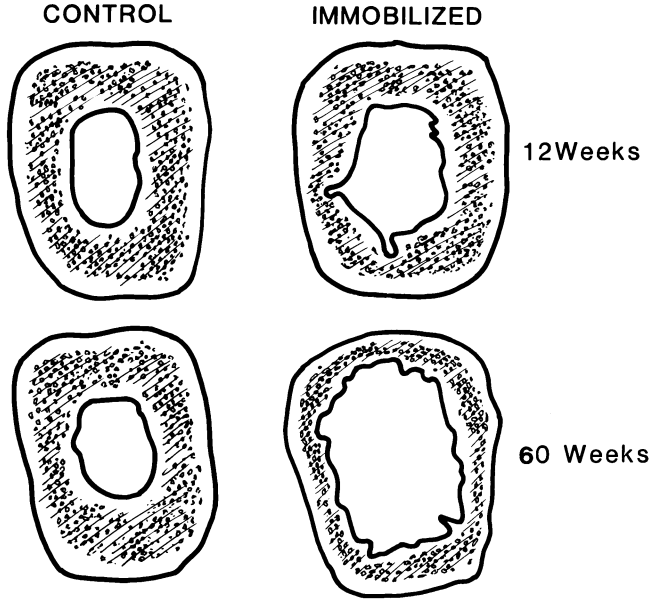


**Figure 4.** Diagram of cross section of the third metacarpus at 12 (**top**) and 60 (**bottom**) weeks of immobilization in old dogs. The control side is **left** and the immobilized side on the **right**. Note the expansion (sloughing of the peri-medullary or peri-endocortical area) of the bone marrow cavity at both time periods and the thinner cortex at 60 weeks. Adapted from Jaworski and Uhthoff[66].

Most of the responses from the immobilization (IM) models in Table 3 are in agreement with the general responses

described above except for those seen in 6-month-old rats with hindlimb suspension[76]. Only periosteal and trabecular bone formation was depressed with no impact on either cancellous and cortical bone mass.

In summary, immobilization or disuse triggers an early (i.e. acute stage) cancellous bone loss that significantly reduces periosteal modeling-dependent bone gain (e.g. inhibits periosteal bone formation) and increases endocortical bone remodeling-dependent bone loss (e.g. BMU creations increase and completed BMUs make less bone than normal) so that trabecular and endocortical bone turnover and net losses increase. The early cancellous bone loss levels off despite continual immobilization (the final or steady state stage) and bone turnover decreases to near normal yet with a permanently reduced bone mass as bone mass and reduced mechanical demand equilibrates. The same acute and steady state also occurs in cortical bone sites but much slower. These tissue-level responses are in accordance with the predictions of Frost's mechanostat theory that suggests when bone biomechanical strains fall and stay below the remodeling threshold as in disuse or immobilization, remodeling predominates in its disuse bone loss mode

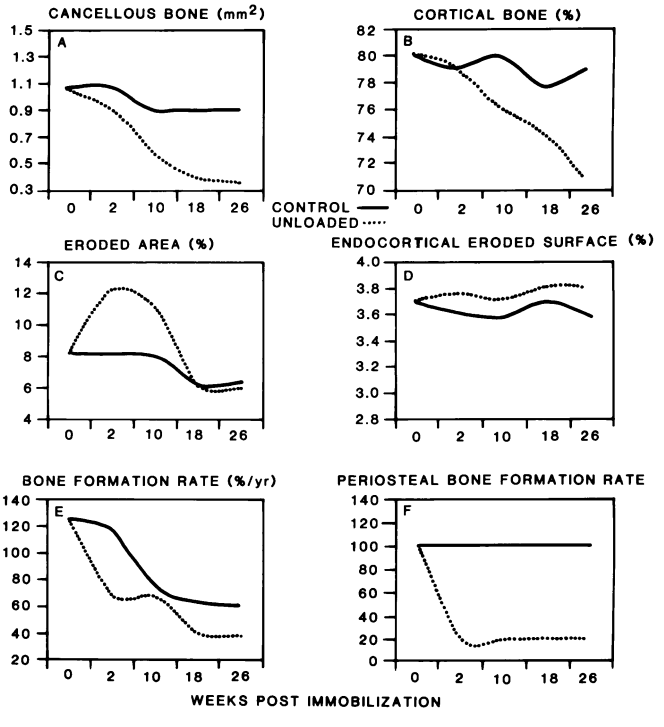


**Figure 5.** Time course changes in cancellous bone in the proximal tibial metaphysis ((PTM, **A**) and cortical bone in the tibial shaft (TX, **B**) of right-hindlimb-immobilized rats. In the PTM (**A**), cancellous bone was 60% at 18 weeks post-IM and plateaued thereafter. The bone loss was mainly due to the negative bone balance from the transient elevation in bone resorption (% eroded area, **C**) and depression in bone formation (**E**). In the TX (**B**), cortical bone loss was limited to 10% after 26 weeks post-IM resulting from the elevation in endocortical bone resorption (**D**) and a dramatic depression in periosteal bone formation (**F**). Adapted from Li et al.[15,74] and Jee et al.[71]

(increase bone turnover and loss) and mechanically controlled modeling stays off and no microdamage arises[82-84]. In contrast to the lack of OVX-induced bone loss in the distal tibial metaphysis (DTM), the immobilization of the hindlimb results in significant bone loss in the DTM[72,73]. Thus, the immobilization-induced distal tibial metaphysis model would be an appropriate model to test especially anabolic agents in the prevention and treatment of osteopenia/osteoporosis in an adult bone site exhibiting low bone turnover.

## The problems or shortcomings of the OVX and IM models

In order to employ the rat OVX and IM models appropriately, investigators must be aware of several shortcomings of the respective models. The apparent problems are:

1. The need for proper controls in dealing with growing bone sites
2. The need for muscle mass or strength data
3. The site specificity of the OVX response
4. The site specificity of the one-legged IM response
5. The manipulation of cancellous bone sites to ensure the prevailing activity is remodeling
6. The lack of Haversian or intracortical remodeling in the rodent
7. The slow developing cortical bone loss

Some of these problems have been readily overcome by altering research designs as selection of sites and endpoints while others have been ignored for the sake of convenience. The next section lists how some of the problems have been handled and suggests solutions for others.

## The solutions

1. The need for proper controls in dealing with a growing bone site

Since the long bones of the rodent skeleton spend the majority of their life span with open epiphyses and continued capacity for bone elongation, there is a need for beginning and/or pre-treatment controls to monitor the effect of growth. It is obvious from a review of the literature in this area that many investigations have failed to include time 0 controls. The omission of these controls can lead to misinterpretation of the skeletal response. One can conclude that cancellous bone loss occurred when in reality it could have been a depression of bone growth or a combination of both.

2. The need for muscle mass measurements

Currently few studies have included muscle mass data in their protocol. The harvesting and weighing of muscles at autopsy is a simple and inexpensive method of obtaining

muscle mass data. Muscle mass or strength measurements are early predictors of bone changes because recent reports demonstrate a strong correlation between different measurements and/or indicators of bone strength and muscle strength[84-93].

3. The site specificity of the OVX response

Not all cancellous bone sites of the rat skeleton lose bone after ovariectomy. The epiphyseal spongiosa, the distal tibial metaphysis and caudal vertebral body are sites known to be resistant to OVX-induced bone loss[3,26,27,29]. Research at these sites could be quite rewarding because these sites are low bone turnover sites and the distal tibial spongiosa is similar to adult human cancellous bone architecturally (i.e. trabecular width, number)[13,26,72,73,87]. To elicit bone loss at these sites one must combine the OVX with the IM model to obtain bone loss[27,56,61,75,94,96]. Table 5 shows that the combination of OVX plus IM can increase the rate of bone loss about 2-fold.

4. The site specificity of the IM-rodent model

A careful inspection of the remaining rat models listed in Table 3 suggests the one hindlimb taping or casting to be the best studied models for IM-induced bone loss[15,57,66-68,71-74]. The taping or casting model is readily reproducible, needs no surgery that sets off the RAP, and static and dynamic histomorphometric data and time course data from slowly growing rats (e.g. from 9 months or older) and adult bone sites (e.g. distal tibial metaphyses) are available and recovery responses[69,72] can be studied. A disadvantage of the taping model is the rats may free themselves frequently. Still the current literature on IM-models leaves much to be desired. There are limited data generated from skeletally mature bone sites in rats, little or no data on the effects of immobilization in the vertebrae and proximal femur (e.g. femoral neck) sites at risk to osteoporosis-induced bone fracture. None of the current models can be readily employed to study the vertebral response due to the lack of understanding as to what occurs at this site following IM. This is made apparent as there is only a single report of the effect of neurectomy on caudal vertebral bone[79]. This report described a reduction in cancellous bone formation rate and a trend toward increased bone resorption. The effects were similar to the effect of mechanical disuse in weight-bearing bones. They concluded that strains associated with normal mechanical usage in caudal vertebrae exert a significant influence on bone formation rate. Whether the one-hindlimb IM taping or casting or possibly nerve resection can be used to immobilize the proximal femur should be explored. Lastly, it is puzzling why the response at the distal tibia immobilization has not been studied. The distal tibia of the rat is an adult bone state at about 3 months of age. The growth plate closes at 3 months[11] with trabecular dynamic histomorphometric profiles similar to adult humans[13,26,72,73,87].

5. The exploitation of specific cancellous bone sites to ensure the prevailing activity is remodeling

The shortcoming of dealing with a growing and not an adult bone site can be overcome. In in vivo osteoporosis research, Kimmel recommenced using the 10-month-old virgin female rat to sample cancellous bone site such as the proximal tibial metaphysis (PTM) and the spongiosa of the lumbar vertebral body (LVB)[5]. The proximal tibia is still growing at less than 3 μm/day and ceases growing at 15 months. In a one-year study, this tibial growth will add less than one mm of new bone. The standard procedure is to begin one's analysis of the secondary spongiosa at one mm distal to the growth plate to avoid any new bone growth as well as to exclude primary spongiosa where trabeculae are modeling and to restrict the analysis to the secondary spongiosa whose prevailing activity is remodeling[6]. By doing so, this will overcome the FDA objection that one is dealing with modeling instead of remodeling activities. When dealing with the LVB, only 0.5 mm from each growth plate should be excluded in order to eliminate the primary spongiosa because the 10-month-old LVB has almost ceased growing[6]. When dealing with an adult bone site like the distal tibia, whose epiphyses is closed at 3 months[11], one should analyze its entire spongiosa beginning just proximal to the sub-chondral bone (Fig. 6).

6. The lack of Haversian or intracortical remodeling in rodents

The FDA guidelines imply that the rat's low levels of

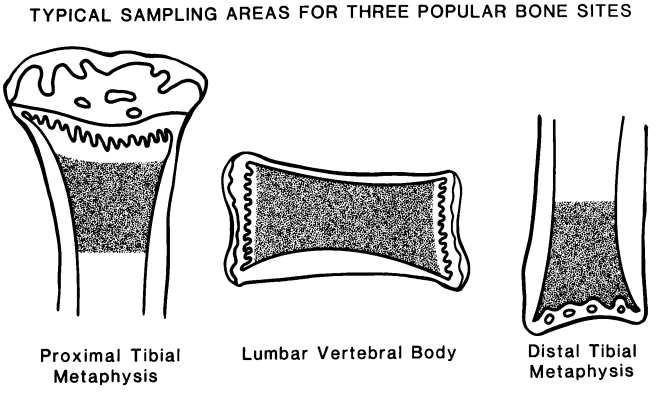


**Figure 6.** Diagram of sampling areas in the proximal tibial metaphysis (PTM), lumbar vertebral body (LVB) and distal tibia (DT) of 6-month-old female rat. In the PTM, analysis should begin one mm distal to the growth plate to avoid involving the primary spongiosa and avoiding all new bone growth. In the LVB, analysis should begin from 0.5mm from each growth plate and in the DT, with its closed growth plate, just proximal from the articular cartilage. Because of the variation in content and architecture in the above sampling areas, more valuable information can be gathered when the area is divided into 3 distal or proximal subzones of 1mm in length for the PTM and DT and in 3 equally divided zones in the LVB. Some investigators do not include cancellous bone immediately adjacent to the endocortical surface.

Haversian remodeling do not permit accurate evaluation of intracortical bone activity, thereby recommending studies of larger animals a requirement in preclinical studies[4]. Unfortunately, up to now, larger animal studies employing the nonhuman primate have not contributed much to our understanding of OVX-induced intracortical bone response. (Also, see section on nonhuman primate ovariectomy model). It is a well-established fact that the bulk in the reduction in cortical bone loss with aging in all species is concentrated in the peri-medullary or peri-endocortical bone adjacent to marrow (Fig. 2). It has been shown that the effects of OVX and IM enlarge the marrow cavity in rats (Fig. 7). Older dogs at 40 weeks of immobilization showed a marked progressive expansion of the marrow cavity (Fig. 4)[15,66-68]. Bone anabolic agents like prostaglandin $E_2$ ($PGE_2$) in rats and parathyroid hormone in nonhuman primates are known to stimulate endocortical bone turnover adjacent to marrow[40,41,97-99]. In addition, the bone added adjacent to marrow by $PGE_2$ is lost with the withdrawal of $PGE_2$ treatment (Fig. 7)[98]. Since the pattern and location of most of the cortical bone loss are identical in rodents and the larger mammals, the information gain in rodent studies may be sufficient to evaluate cortical bone behavior without involving expensive large animal studies.

7. The slow developing cortical bone loss.

In Tables 2 and 4, it is shown that both the OVX- and IM-induced cortical bone loss is slow to develop. The earliest sign of any bone resorption is indirectly by the expansion of the medullary cavity at 42 and 90 days for the IM and OVX models, while only the IM model cortical bone loss occurs by 70 days. The cessation of bone loss is not well established and is estimated to be more than 180 days[15]. The published data leave much to be desired in that no time course studies have been reported for OVX-induced cortical bone loss. All such studies should employ a more sensitive approach to evaluate the effects of OVX and IM models employed by several investigators. Since the bulk of the OVX- and IM-induced bone resorption occurred at the endocortical surface adjacent to marrow, one should employ the method of Danielsen et al.[35] in which they determined the cortical width of the inner half of the femoral shaft adjacent to the marrow. This approach focuses the analysis to the site where the bulk of bone loss occurs. Burr and co-workers have divided the shaft of larger animals into three zones, an outer, middle and inner zone to direct regional distribution of bone remodeling in larger animals (Fig. 8)[99,101]. Since the long bone in smaller animals like the rat are smaller and often irregular, the approach depicted in Figure 9 can be used to improve detecting cortical bone loss. Using a similar technique, Danielsen et al.[35] reported the reduction in inner cortical thickness by 10% at 6 months post OVX. Besides determining the content of peri-medullary or peri-endocortical bone, one can accelerate the rate of cortical bone loss by combining OVX and IM into one model[27,56,61,75,94,96]. Okumura et al.[56,61] reported an additive reduction in femoral score with the combination of IM plus OVX treatment (IM + OVX, -28% vs. OVX, -5% and IM, -11% at 12 weeks) (Table 5). The femoral score is the bone width minus the medullary width divided by the bone width x 100. Their IM procedure used hemichordectomy and sciatic resection surgery to produce the immobilization, a technique that most animal use committees would not allow. This is unfortunate because a time course study of the combined models could be most informative and compresses

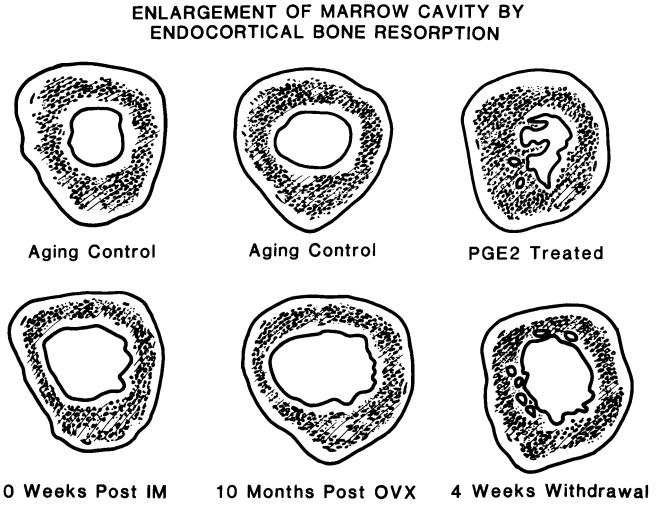


**Figure 7.** Diagram of tibial shaft at the tibio-fibular junction showing the loss of perimedullary bone after immobilization (IM, **left**), ovariectomy (OVX, **middle**) and after withdrawal of $PGE_2$ treatment (withdrawal, **right**) and their respective controls (**top row**). Adapted from Jee et al.[98], Li and Jee[15] and Tang et al.[100]

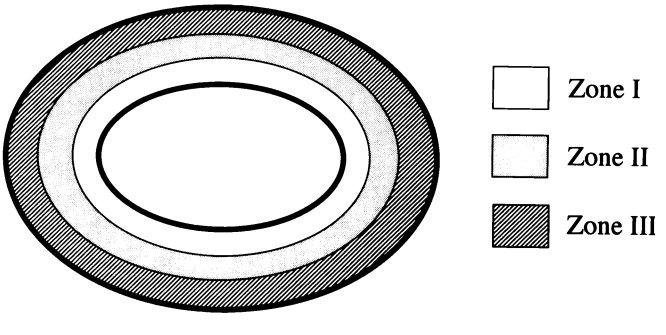


**Figure 8.** Diagram of subdivision of the cortex to increase the sensitivity of detecting cortical bone loss originating adjacent to marrow and cortical bone proper. The femoral shaft in larger animals like a dog or rabbit, one can be readily divide it into three zones by measuring cortical diameter microscopically and dividing it equally into three parts. Zone I was adjacent to endocortical surface, Zone II was intermediate and Zone III was near the periosteal surface. Cortical area, thickness and porosity could then be measured in each zone to evaluate the distribution of these parameters. From Burr et al.[99] Reproduced from J Bone Miner Res 2000:157-165, with permission of American Society for Bone and Mineral Research.

| Bone tissue | Cancellous bone of proximal tibial metaphyses | | Cortical bone of femoral shaft | |
|---|---|---|---|---|
| **Treatment** | **56 days** [96] | **84 days** [61] | **84 days** [56] | **168 days** [56] |
| Ovariectomy (OVX) | - 60%* | -- | - 5% | - 10% |
| Hemichordectomy | -- ** | -- | - 11% | - 16% |
| Hemichordectomy+OVX | -- | - 90% | - 28% | - 47% |
| Sciatic resection | -- | -- | 0 | - 10% |
| Sciatic resection+OVX | -- | - 77% | - 11% | - 28% |
| Taping | - 44% | -- | -- | -- |
| Taping+OVX | - 80% | -- | -- | -- |
| * percent of controls, ** -- no determinations | | | | |

**Table 5.** Comparison of the responses of immobilization (IM) [Hemichordectomy, Sciatic Resection & Taping] with ovariectomy (OVX) and OVX and IM alone.

the time needed to do a restoration study.

Both J. Gasser and R. Erben (personal communications) have stated that the generation of significant rapid ovariectomized-induced cortical bone loss is not a problem, it occurs rapidly in older rats. Nevertheless, such data is missing in the open literature.

## Other osteopenia/osteoporosis models

Other animal species that may contribute to osteoporosis research include the OVX'd nonhuman primate, OVX'd mouse, the senescence accelerated mouse (SAM/P6) and the glucocorticoid-induced bone loss in the mouse.

## The nonhuman primate ovariectomized model

Recent recommendations and draft guidelines for drug registration require that agents for prevention and treatment of postmenopausal osteoporosis be tested in the ovariecto-mized rat model and one larger bone remodeling species[4,102]. The requirement of a larger remodeling species is due to a prevailing opinion that rat bone does not remodel and that larger animals display Haversian remodeling. Relatively few studies of the effect of ovariectomy have been done in larger species, including dogs, pigs, sheep, ferrets and nonhuman primates. More studies have been done in nonhuman primates than in any other species except rats and mice and those studies have consistently demonstrated development of osteopenia accompanied by high bone turnover rates after ovariectomy. In monkeys ovariecto-mized for 2 years, spinal osteopenia ranged from 11% to 15% lower mean bone mass in ovariectomized animals than in intact animals. Whether sufficient osteopenia occurred needed classification[103,104]. Bone turnover rates were increased for up to 2 years in ovariectomized monkeys as evidenced by increased serum and urine markers[103-106] and increased bone formation rates measured histomorphometrically[103,105-109]. These changes resemble that in postmenopausal women, therefore many investigators have preferred the estrogen-depleted nonhuman primates as the large animal of choice. Further validation of the ovariectomized nonhuman primate models include demonstrating of absolute osteopenia using dual-energy X-ray absorptiometry[109,110] and decreased bone strength using biomechanical testing of the spine and femoral neck[111-113].

Recently the detailed changes in the cortical bone of the humeral and tibial shaft in adult ovarectomized cynomolgus monkeys treated with one and 5 μg PTH (LY333334)/kg/d for 18 months have been reported[99]. The number of resorption cavities, activation frequency and bone volume based bone turnover was increased 75%, 227% and 333% respectively. Cortical porosity was significantly increased due mainly to an increased porosity in the inner third of the cortical diameter (25% in treated versus 5% in OVX and Sham controls). Cortical thickness was decreased but no difference in cortical area, medullary area and bone area as well as for strength (ultimate force, stiffness or work to fracture).

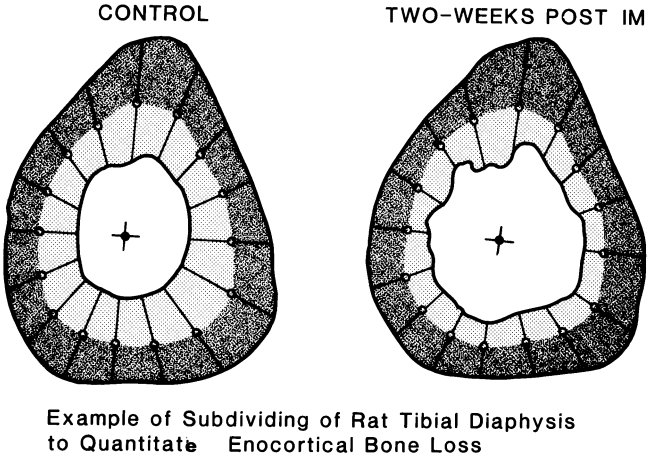


**Figure 9.** Diagrammatic example of subdividing the irregular-shaped tibial shaft in smaller animals like the rat to increase the sensitivity for determining cortical bone loss adjacent to marrow. First determine the area of the outer zone (one half of cortical thickness, dark shading) in controls and duplicate this outer zone in the treated specimen (**right**). The inner zone (less shading) in the treated rat now shows a significant reduced area of cortical bone due to immobilization-induced cortical bone loss occurring at the endocortical surface adjacent to marrow.

There are some of us who feel that there is no need for a larger remodeling ovariectomized species. The reason for a larger species is that many claim the rat is not a bone remodeling species. On the contrary, it has been shown that similar to higher mammals the prevailing activity in vertebral and tibial cancellous bone of aged (12-month-old) rats is remodeling[6]. Even the cortical bone proper in the rat displays low levels of intracortical remodeling and the prevailing activity at the endocortical surface are remodeling[6,7]. The latter activity is important because ovariectomy decreases cortical thickness and porosity in the inner third of the cortex in both rats and larger species by endocortical bone resorption (by remodeling-induced bone loss adjacent to marrow). Since anabolic agents are known to stimulate cortical bone and increased cortical porosity in the inner one third of the cortical diameter, no significant reduction in strength may occur[7,40,97-99]. If porosity were uniformly distributed throughout the cross section of the cortex, the reduction in strength of the bone would have been greater than when the porosity is primarily distributed adjacent to the endocortical surface[99]. Since bone strength has been tested in bone sites at fracture risk in osteoporotic humans in the rat, such studies in nonhuman primates would only be confirmatory. In summary, there is no new information forthcoming from a nonhuman primate study that cannot be obtained from a well-designed rat ovariectomy study; therefore there is no need for time consuming, expensive studies of this larger species.

## The ovariectomized mouse model

Data to validate the ovariectomized mouse as an in vivo model for osteoporosis research per se are in short supply. All the publications dealing with this model have been to study the short-term effects of cytokines and hormones. Bain and colleagues (personal communication) have actually done considerable work in mice that isn't published. They stated the time course in the proximal tibial metaphysis is essentially the same as the rat; in a 5 week study of Swiss-Webster mice, they will lose 50% of their cancellous bone mass[114-116]. The time course of cortical bone changes are probably the same as the rat except that up to now there have not been studies long enough to see changes[117]. In addition, the incidence of bone remodeling vs. modeling in cancellous bone is unknown. All the publications thus far dealt with very young mice (8 weeks old) and until it is shown that the older mouse has the similar time course and site specificity for the development of estrogen depletion osteopenia/ osteoporosis in a strain specific fashion as it has been for the rat, few investigators will be induced to choose the ovariectomized mouse model. Nevertheless, the ovariectomized mouse can be useful as an initial in vivo screening of new drug candidates since much less drug is needed. Of course the next step is to employ the rat for evaluation of bone efficacy of selected lead compounds.

## The senescence accelerated mouse (SAM/P6) model

The senescence accelerated mouse (SAM/P6), a mouse model for severe osteoporosis[1] has low peak bone mass and develops fractures in old age[118-123]. Bone development is normal during the first 3 months, but osteopenia progressively develops thereafter[118,124]. The predictable occurrence of osteopenia/osteoporosis makes the SAM/P6 mouse a unique model for study of age-related osteopenia and severe osteoporosis. Manolagas and Jilka[125] proposed the reduction in osteoblastogenesis in SAM/P6 mice is due to a change in the direction of differentiation of a common progenitor away from the osteoblast lineage in favor of adipocytes[124-126]. They conclude the behavior of the bone and bone marrow in 4 month and older SAM/P6 mice mimics many aspects of the age-related changes seen in bones of humans. Because these mice provide a faithful model of age-related osteopenia in humans, they provide the opportunity to identify relevant genes that contribute to this process.

## The mouse glucocorticoid treated model

Weinstein and Manolagas[127,128] have demonstrated that the mouse can be a reliable animal model of glucocorticoid-induced osteopenia/osteoporosis and mimic the changes seen in humans. Mice receiving glucocorticoid for 7 days showed an early increase in bone resorption and exhibited at week 4 decreased bone mineral density; numbers of osteoblasts and osteoclasts, progenitors in the bone marrow, osteoid area, mineral appositional rate, bone formation rate and a dramatic reduction in cancellous bone mass. In addition, glucocorticoid administration caused a 3-fold increase in osteoblast apoptosis in vertebrae and 28% osteocytic apoptosis in metaphyseal cortical bone. Missing again is the need for longer time course and site specificity studies for the development of glucocorticoid-induced osteopenia/osteoporosis in a fashion done for the ovariectomized rat. Nevertheless, this model, if reproduced by others, is an exciting breakthrough of having an animal model to study agents to prevent or treat glucocorticoid-induced osteoporosis.

## Summary

Prior to initiating a clinical trial in post-menopausal osteoporosis study, it is reasonable to recommence the evaluation of treatment in the 9-month-old ovariectomized female rat. A female rat of this age has reached peak bone mass and can be manipulated to simulate clinical findings of post-menopausal osteoporosis. Ample time exists for experimental protocols that either prevent estrogen depletion osteopenia or restore bone loss after estrogen depletion. Methods like serum biochemistry, histomorphometry and densitometry used in humans are applicable in rats. Like most animal models of osteopenia, the rat

develops no fragility fractures but mechanical testing of rat bones substitute as a predictor of bone fragility. Recent studies have shown that the prevailing activity in cancellous and cortical bone of the sampling sites in rats is remodeling. The problems of dealing with a growing skeleton, the site specificity of the OVX and IM models, the lack of trabecular and Haversian remodeling and the slow developing cortical bone loss have been and can be overcome by adding beginning and pre-treatment controls and muscle mass measurements in all experimental designs, selecting cancellous bone sampling sites that are remodeling, concentrating the analysis of cortical bone loss to the peri-medullary bone and combining OVX and IM in a model to accelerate the development of cortical bone osteopenia. Not to be forgotten is the distal tibia site, an adult bone site with growth plate closure at 3 months and low trabecular bone turnover and architecture similar to human spongiosa. This site would be most challenging to the action of bone anabolic agents. Data about estrogen-deplete mice are encouraging, but the ovariectomized rat model suggests that developing an ovariectomized mouse model as an alternative is not urgent. When the exciting mouse glucocorticoid-induced bone loss model of Weinstein and Manolagas[127,128] is combined by others, it could be a significant breakthrough for that area of research. Lastly, the information generated from skeletal studies of nonhuman primates has been most disappointing and since much of the older rat skeleton is remodeling, I recommend that these expensive large animal skeletal studies be curtailed unless it is required by a regulatory agency for safety studies.

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