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Osteoporosis: An Overview

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Abstract

Osteoporosis is a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue. Approximately 8 million women and 2 million men in the United States have osteoporosis, and 34 million persons have osteopenia. Primary osteoporosis is the result of bone loss related to the decline in gonadal function associated with aging. Secondary osteoporosis may result from chronic diseases, exposures, or nutritional deficiencies that adversely impact bone metabolism. Various factors are known to influence bone mass accumulation during growth which include genetic factors, endocrine factors (sex steroids, calcitriol, insulin-like growth factor-I (IGF-I)), mechanical forces (physical activity, body weight). The ideal screening method to characterize bone status would include parameters of bone density, bone microarchitecture, markers of bone formation and marks of bone resorption. The favored methods of bone evaluation are DEXA, CXD and one another low cost method of screening in which skin fold thickness (SFT) is measured over the fourth metacarpus with Holtain Tanner Whitehouse calipers. Non pharmacological and pharmacological treatment may improve the bone loss in osteoporosis. The biological and biomechanical characteristics of orthopaedic implants, bone-graft substitutes (with or without osteogenic bone morphogenetic proteins) can be tested on large numbers of animals maintained with a level of experimental control, impossible in human clinical research. Various animal models mainly used for osteoporosis are immobilized rat model, nonhuman primate ovariectomized model the ovariectomized mouse model, the senescence accelerated mouse (SAM/P6) model**,** the mouse glucocorticoid treated model.

Key Words: Osteoporosis, Microarchitecture, Osteoporotic models

Introduction

Osteoporosis is a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue. At a given age, bone mass results from the amount of bone acquired during growth, i.e. the peak bone mass[1,2] minus the age-related bone loss which particularly accelerates after menopause. The rate and magnitude of bone mass gain during the pubertal years and of bone loss in later life may markedly differ from one skeletal site to another, as well as from one individual to another. Bone mass gain is mainly related to increases in bone size that is in bone external dimensions, with minimal changes in bone microarchitecture. In contrast, postmenopausal and age-related decreases in bone mass result from thinning of both cortices and trabeculae, from perforation and eventually disappearance of the latter, leading to significant alterations of the bone microarchitecture (figure 1). Approximately 8 million women and 2 million men in the United States have osteoporosis, and 34 million persons have osteopenia[3]. A bout one in two white women will experience an osteoporotic fracture in her lifetime[4,5]. Osteoporosis also occurs in older men, who have a higher mortality from hip fractures and a lower frequency of screening and treatment[6,7].

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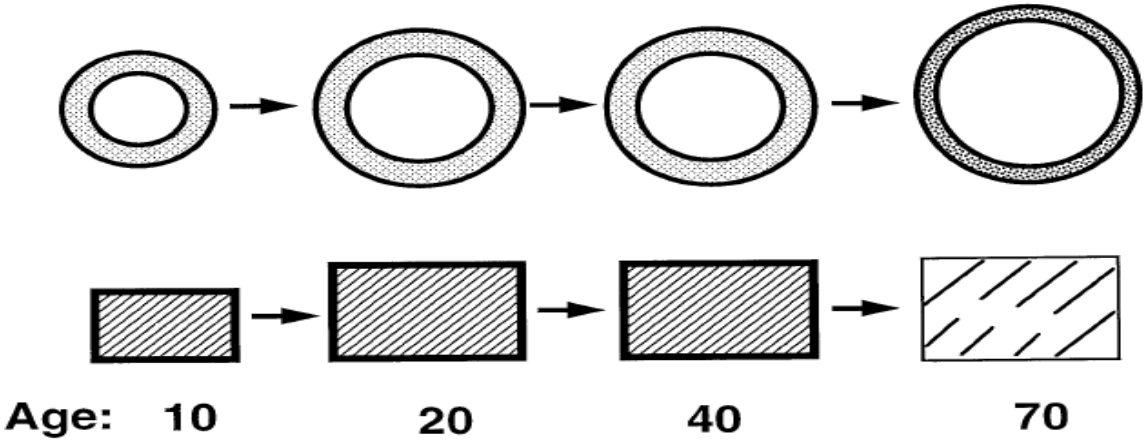
Figure No. 1: Schematic representation of cortical and cancellous bone changes throughout life. Stippling represents cortical porosity and hatching represents cancellous bone network



Overall, hip fractures cause an excess mortality of 10 to 20 percent at 12 months, and up to 25 percent of patients with hip fractures require long-term nursing home care[4]. In 2002, the cost of a hip fracture was estimated to be $34,000 to $43,000, with the annual cost of all osteoporotic fractures as high as $18 billion[3]. Despite broadly accepted screening, diagnosis, and treatment guidelines, there is a large gap between knowledge and effective clinical practice. O ne study showed that only 49 percent of women were evaluated or treated in accordance with accepted guidelines[7].

The World Health Organization (WHO) defines osteoporosis as a spinal or hip bone mineral density (BMD) of 2.5 standard deviations or more below the mean for healthy, young women (T-score of −2.5 or below) as measured by dual energy x-ray absorptiometry (DEXA)[8]. Osteopenia is defined as a spinal or hip BMD between 1 and 2.5 standard deviations below the mean[5,8]. Primary osteoporosis is the result of bone loss related to the decline in gonadal function associated with aging[8]. Selected factors that are associated with fracture or low BMD are listed in Table No. 1[4,5]

Table No. 1: Selected Factors Associated with Fracture or Low Bone Mineral Density in Postmenopausal Women

1	Increasing age	8	Excessive alcohol (> 2 drinks per day),
2	Low body weight (< 127 lb [58 kg])	9	caffeine, and tobacco use
3	Personal history of fracture	10	History of falls
4	Family history of osteoporotic	11	Low level of physical activity
5	fracture	12	Low calcium or vitamin D intake
6	Not using hormone therapy	13	Use of certain medications
7	White or Asian race	14	Presence of certain medical conditions

Secondary osteoporosis may result from chronic diseases, exposures, or nutritional deficiencies that adversely impact bone metabolism. Causes of secondary osteoporosis are listed in Table No. 2[3,4,8,9].

Table No. 2: Causes of Secondary Osteoporosis

Cause	Examples	Cause	Examples
Chronic medical and systemic diseases	• Amyloidosis	Medication	• Anticonvulsants (e.g., phenobarbital,

	• Ankylosing spondylitis • Chronic obstructive pulmonary disease • Human immunodeficiency virus • Acquired immunodeficiency syndrome • Inflammatory bowel diseases • Liver disease (severe) • Multiple myeloma • Renal insufficiency or renal failure • Rheumatoid arthritis • Systemic lupus erythematosus		phenytoin[Dilantin]) • Drugs causing hypogonadism (e.g., parenteral progesterone, methotrexate, gonadotropinreleasing hormone agonists) • Glucocorticoids • Heparin (long-term) • Immunosuppressants (e.g., cyclosporine [Sandimmune], tacrolimus [Prograf]) • Lithium • Thyroid hormone excess
Endocrine and metabolic disorders	• Athletic amenorrhea • Cushing syndrome • Diabetes mellitus, type 1 • Hemochromatosis • Hyperadrenocorticism • Hyperparathyroidism (primary) • Hyperthyroidism • Hypogonadism (primary and secondary) • Hypophosphatasia	Nutrition	• Alcohol (> 2 drinks per day) • Anorexia nervosa • Celiac disease • Gastric bypass or gastrectomy • Vitamin D deficiency

Endocrine regulation of bone mass

Many factors, more or less dependent on each other, are known to influence bone mass accumulation during growth. These determinants classically include genetic factors, which quantitatively appear the most prominent factors[10,11], race, gender, nutrients (calcium, protein, phosphate), endocrine factors (sex steroids, calcitriol, insulin-like growth factor-I (IGF-I)), mechanical forces (physical activity, body weight), and exposure to risk factors[12,13]. Most of these factors are also involved in the maintenance of bone mass during adulthood as well as in bone loss later in life, although in variable proportions compared with their role in peak bone mass acquisition.s

The vitamin D system

Vitamin D_3 is for the most part synthesized from its 7-dehydrocholesterol precursor in the dermis under ultra violet B radiations. It is sequentially hydroxylated by liver and kidneys in its hormonal metabolite, calcitriol, i.e. 1,25-dihydroxyvitamin D (1,25(OH)2 D3). The effects of ($1,25(OH)_2$ D_3) are mediated by its nuclear vitamin D receptor (VDR). Upon binding of ($1,25(OH)_2$ D_3), VDR forms a heterodimeric complex with the retinoic acid receptor and additional transcription factors, and ultimately regulates the expression of a number of genes bearing vitamin D responsive elements in their promoter region[14,15]. The role of vitamin D metabolites is primarily to maintain serum calcium and phosphate levels by directly promoting intestinal absorption of these ions as well as by activating bone resorption[16]. Failure of the vitamin D endocrine system during growth causes rickets, which is a prominent bone-

deforming and sometimes life-threatening disorder. Vitamin D is also important in the maintenance of skeleton integrity in adults. Elderly people tend to have poor dairy calcium and vitamin D intakes, decreased sunlight exposure and dermal production of vitamin D, and diminished production of ($1,25(OH)_2$ D_3) with secondary hyperparathyroidism. In turn, vitamin D and calcium supplementation has been demonstrated to significantly increase BMD and decrease the incidence of osteoporotic fractures in the elderly[17,18,19].

Estrogens

Female sex hormones appear to be mandatory, not only for the acquisition of peak bone mass in both females and males[20,21,22], but also for the maintenance of bone mass in adults. They control bone remodeling during reproductive life in females[23,24] and later on in aging men[25,26]. Pathologic conditions associated with premature estrogen deficiency, such as anorexia nervosa, secondary amenorrhea due to strenuous exercise, or the use of inhibitors of gonadotropin secretion, further support the concept of a causal link between estrogen deficiency and accelerated bone loss[27,28]. By indirectly accelerating bone turnover and by uncoupling bone formation from resorption[29], estrogen deficiency is the main cause of postmenopausal osteoporosis, and possibly plays an important role in male osteoporosi as well[30]. Thus, estrogen deficiency is directly implicated in the age-related increase in the incidence of fragility fractures[24]. In addition, estrogen deficiency also seems to be correlated with the progressive increase in serum parathyroid hormone (PTH) levels observed in aging individuals, which by itself contributes to accelerate bone turnover[31].

IGF-I

IGF-I is an essential factor for longitudinal bone growth[32], as it stimulates proliferation and differentiation of chondrocytes in the epiphyseal plate[33]. IGF-I also plays a role in trabecular and cortical bone formation. This factor can stimulate both proliferation and differentiation of osteoblasts; it increases type I collagen synthesis, alkaline phosphatase activity and osteocalcin production[34]. Thus, IGF-I can exert anabolic effects on bone mass not only during growth, but also during adulthood[35,36,37]. Furthermore, by its renal action on tubular reabsorption of phosphate and on the synthesis of calcitriol, through a direct action on renal cells[38,39], IGF-I can be considered as an important controller of the intestinal absorption and of the extracellular concentration of both calcium and phosphate, the main elements of bone mineral. On the other hand, IGF-I can selectively stimulate the transport of inorganic phosphate across the plasma membrane in some osteoblastic cell lines[40]. Osteogenic cells not only express specific IGF-I receptors, but they can also be endowed with IGF-I producing machinery[33,41]. Taking into account these experimental and clinical observations, IGF-I could play a prominent role in the pathophysiology of osteoporosis, of osteoporotic fracture and of its complications. In association with age, several reports have documented a decrement in IGF-I plasma levels[42,43,44,45]. Under these conditions, a restoration of this altered system in the elderly, for instance by protein replenishment[46], is likely to favorably influence not only BMD, but also muscle mass and strength, since these two variables are important determinants of the risk of falling[47].

Thus, bone mass gain during childhood, bone loss after menopause and further loss in the elderly are determined by different sets of endogenous and exogenous factors[48,49], and the relative influence of specific genes on the risk of osteoporosis may vary greatly with age. We will now examine the genetic aspects of hormones and their receptors involved in the regulation of bone accumulation and loss.

Screening for osteoporosis

The ideal screening method to characterize bone status would include parameters of bone density, bone microarchitecture, markers of bone formation and marks of bone resorption[50]. Currently, on one cost effective screening methods have been developed to measure all four markers of bone health[50,51,52]. Development of a low cost screening tool that efficiently can identify women at risk would enable healthcare professionals to place greater emphasis on prevention and intervention in this subset of the population.

Currently, dual energy X-ray absorptiometry (DEXA), with traditional skeletal X-rays when indicated, are the favored methods of bone evaluation; however, there are no data that evaluate the cost effectiveness of DEXA for mass screening[50]. Because of the association of bone mass in those with osteoporosis with 100% increased risk of fracture and the reproducibility of DEXA bone measurements, the use of protable DEXA units that would allow for greater flexibility and possibly greater cost saving in screening large populations of women has been suggested[53,54]. Although one-site scanning would be the most cost-effective DEXA screen, the location of the most sensitive area for evaluation has not been determined.

X-ray densitometry (CXD), a semiautomated method of radiographic densitometry, is another promising method of osteoporosis screening. In CXD, a radiograph of the second metacarpal bone is taken with reference to an aluminum phantom. Yamamoto et al.[55] found that CXD was especially useful in screening patients with osteoporosis or women older than age 70, because degenerative diseases of the lumbar spine in this group may produce skewed bone mineral density values when DEXA screening is used.

Biochemical markers for bone formation and resorption also are used to assess bone health[50,52]. Markers of bone formation include total and bone specific serum alkaline phosphatase, serum osteocalcin and serum type I collagen extension peptides. Bone resorption markers include urinary hydroxyproline, urinary excretion of pyridinium cross-links and the measurement of plasma tartrate-resistant acid phosphatase activity. Urinary pyridinoline and serum osteocalcin have been clinically useful in screening bone turnover in menopausal women and in assessing the level of bone turnover in elderly women with vertebral osteoporosis[52]. A method that is becoming widely used for population-based screening of vertebral osteoporosis is the measurement of the urinary pyridinium cross-links. The excretion of these cross-links is higher in osteoporotic individuals than in those without osteoporosis[56].

Measuring skin fold thickness (SFT) over the fourth metacarpus with Holtain Tanner Whitehouse calipers is a simple, low cost method of screening for osteoporosis. In one study in which this method was used, mean SFT was lower in the osteoporotic women than in normal, nonosteoporotic women. Moreover, a negative correlation between SFT and chronologic and menopausal age was observed in those with osteoporosis but not in normal controls. Including a measurement of SFT as part of a woman's annual examination across the life cycle is a promising method of identifying high-risk group[57]. Serial measurement of bone mineral density and estimates of the rate of bone turnover, using bone markers can be used in high-risk patients identified by SFT in order to assess response to therapeutic interventions[58].

Indications for treatment

Recommendations about which persons with osteoporosis should receive treatment vary[4,8]. T he NOF recommends treatment of postmenopausal women and men with a personal history of hip or vertebral fracture, T-score of –2.5 or below, or low bone mass (T-score between –1 and –2.5) and a 10-year probability of hip fracture of at least 3 percent or any major fracture of at least 20 percent[4]. T he 10-year probability of fracture is calculated using the WHO fracture risk assessment tool (http://osteoed.org/tools.php). The WHO recommendations are less specific, and they differ from those of the NOF. The WHO recommends treating persons with or at risk of osteoporosis. Table No. 3 summarizes prescribing and cost information for medications approved by the U.S. Food and Drug Administration (FDA)[59].

Non pharmacologic treatment

Nutrition

Good nutrition from infancy through adolescence, with particular attention to adequate daily intake of calcium and vitamin D, is a key component for the attainment of maximum PBM. Nutritional disorders known to impair bone accretion in adolescence include anorexia nervosa[60], inflammatory bowel disease, celiac disease, and cystic fibrosis[61]. In reviews of 19 placebo-controlled studies looking at the relationship between calcium intake and bone loss, 16 showed that calcium prevented or slowed bone loss[62,63]. In a recent meta-analysis of randomized trials in postmenopausal women, representing 1,806 participants, it was found that calcium was more effective than placebo in reducing rates of bone loss after two or more years of treatment[64]. The recommended daily intake of elemental calcium for postmenopausal women is 1200 mg[65], which is much more than the average daily intake in this population[66,67,68]. Vitamin D is important for absorption of calcium and mineralization of bone[69], as well as for optimal muscle function and balance[70]. Vitamin D deficiency or insufficiency, defined as a blood level less than 20 or 30 ng/ml, respectively, is common, especially in the frail elderly[71]. While it is often difficult to distinguish the effects of calcium and vitamin D in clinical trials, some studies have shown an increase in BMD and reduction in fracture risk in elderly patients supplemented with calcium and vitamin D[72,73]. Recommended daily intakes of vitamin D may not be adequate to attain optimal blood levels in all patients. When it is necessary to determine adequacy of vitamin D with certainty, serum 25-OH-vitamin D, not 1,25- dihydroxyvitamin D, should be measured. A recent report from the Women's Health Initiative (WHI) demonstrated that calcium and vitamin D supplementation increased hip BMD in the entire cohort of postmenopausal women, and reduced the risk of hip fracture in those who were adherent to therapy, taking estrogen, or age 60 and older[74]. Adequacy of calcium and vitamin D should be assured in all patients, especially those with osteoporosis.

Physical activity

Observational, retrospective, and prospective randomized studies have shown beneficial effects of exercise on bone accumulation during growth, with particular benefit from high impact exercise[75,76]. Excessive exercise can be harmful to skeletal health, as seen in adolescents and young-adults with female athlete triad (disordered eating, amenorrhea, and osteoporosis)[77]. Weight-bearing exercise is associated with small but significant improvement in BMD in premenopausal and postmenopausal women[78] and in men[79]. The Surgeon General recommends a "minimum of 30 minutes of physical activity (such as brisk nwalking) on most, if not all, days of the week"[80].

Other lifestyle factors

Cigarette smoking and excess alcohol intake should be discouraged during childhood due to well known adverse effects on multiple organ systems[65]. Metaanalyses have shown that cigarette smoking is associated with reduced BMD[81] and increased risk of fracture[82]. Every effort should be made to discourage initiation or continuation of cigarette smoking. Excess alcohol is detrimental to skeletal health for many reasons[83], although moderate alcohol drinking has been associated with higher bone mass in some studies[84,85]. Administration of drugs that are known to be harmful to skeletal health, such as glucocorticoids and anticonvulsants, should be avoided or minimized in dose and duration.

Falls

The vast majority of hip fractures, most other nonvertebral fractures, and some vertebral fractures, occur as a result of a fall. A fracture occurs when the force applied to the bone exceeds the strength of the bone. Prevention of falling is a key component of a fracture prevention program. Weight-bearing exercise with special attention to quadriceps muscle strengthening should be encouraged. Patients can do balance-training independently, with the help of a physical therapist, or through instructional classes in activities such as Yoga or Tai-Chi. Vitamin D supplementation may increase muscle strength, improve balance, and reduce the risk of falls. Hip protectors, if worn regularly, may reduce the risk of hip fractures in patients who are at high risk of falling.

Pharmacologic treatment

Bisphosphonates

Oral bisphosphonates inhibit osteoclastic activity and are potent antiresorptive agents. R andomized clinical trials demonstrate a reduction of vertebral and hip fractures with alendronate (Fosamax)[86,87,88] and risedronate (Actonel)[87,88,89,90]. Alendronate and risedronate have also demonstrated effectiveness in men[91,92] and in glucocorticoid-induced osteoporosis.[93,94] Both daily and intermittent uses of ibandronate (Boniva) have demonstrated antifracture effectiveness at the spine only[88,95]. As age increases, the NNT to prevent all types of fractures decreases[96]. Weekly and monthly dosing make taking bisphosphonates easier. Nevertheless, nonadherence is problematic and is associated with worse outcomes[97]. Oral bisphosphonates must be taken with a full glass of water. A 30 to 60 minute wait is required before reclining or consuming other medications, beverages, or food to lower the risk of upper gastrointestinal adverse effects. The optimal length of oral bisphosphonate therapy is unknown. A recent study found that women who take alendronate for five years followed by five years of placebo have no increase in the incidence of nonvertebral or hip fractures compared with women who take alendronate for 10 years. There is, however, an increase in vertebral fractures[98]. This suggests that relatively low-risk women (i.e., no personal history of vertebral fractures and only modestly reduced T -score) may consider an interruption in bisphosphonate treatment. The intravenous bisphosphonates currently approved by the FDA for the treatment of postmenopausal osteoporosis are zoledronic acid (Reclast), given 5 mg yearly (shown to decrease vertebral and hip fractures)[88,99], and ibandronate, given 3 mg every three months (shown only to increase BMD in the intravenous form; the oral form has been shown to decrease vertebral fractures)[100]. Although the cost of these medications is high, use may prove to be an attractive strategy for high-risk patients who are unable to tolerate or are noncompliant with oral therapy, or those currently hospitalized for hip fracture.

Recent concerns have been raised about the association of bisphosphonates with osteonecrosis of the jaw. To date, this rare complication is most often associated with the frequent infusion of intravenous bisphosphonates in patients with cancer[101].

Table No. 3: Medications Approved by the U.S. Food and Drug Administration for Osteoporosis

Indication	Medication	Typical dosage	Route	Monthly cost*
Prevention	Estrogen, with or without progesterone	0.625 mg daily	Oral	With progesterone: $40 Without progesterone: $47
Prevention and treatment	Alendronate (Fosamax)	70 mg weekly	Oral	Tablet: $87, $77 (generic) Solution: $96
	Ibandronate (Boniva)	150 mg monthly	Oral	$100
	Risedronate (Actonel)	35 mg weekly	Oral	$92
	Raloxifene (Evista)	60 mg daily	Oral	$108
Treatment	Ibandronate	3 mg every three months for four doses	Intravenous	$162
	Zoledronic acid (Reclast)	5 mg annually for three doses	Intravenous	$104
	Calcitonin (Miacalcin)	200 IU daily	Nasal	$126
	Teriparatide (Forteo)	20 mcg daily up to two years	Subcutaneous	$675

Raloxifene

Raloxifene (Evista), a selective estrogen receptor modulator, is approved for the treatment of postmenopausal osteoporosis. Raloxifene has estrogen agonist activity on the bones and lipids, and an estrogen antagonist effect on the breast and uterus. Raloxifene is effective for reducing the incidence of vertebral fractures, but effectiveness at the hip has not been shown[87,88,102]. R aloxifene is commonly associated with increased vasomotor symptoms. Although raloxifene increases the risk of venous thromboembolism, it is indicated to decrease the risk of invasive breast cancer in postmenopausal women with osteoporosis. Perhaps it may be best used in postmenopausal women with osteoporosis who are unable to tolerate bisphosphonates, have no vasomotor symptoms or history of venous thromboembolism, and have a high breast cancer risk score.

Calcitonin

Calcitonin nasal spray (Miacalcin) is an antiresorptive agent approved for the treatment of postmenopausal osteoporosis at a dosage of 200 IU in alternating nostrils each day. It is shown to decrease the occurrence of vertebral compression fractures, but not non vertebral or hip fractures[88,103]. Although calcitonin has modest analgesic properties in the setting of acute and chronic vertebral compression fracture[104], it is not considered first-line treatment for osteoporosis because more effective medications are available.

Teriparatide

Teriparatide (Forteo) is a recombinant human parathyroid hormone with potent bone anabolic activity. In a dosage of 20 mcg per day given subcutaneously for up to two years, teriparatide decreases vertebral and nonvertebral fractures[88,105]. Adverse effects may include orthostatic hypotension, transient hypercalcemia, nausea, arthralgia, and leg cramps. Increased risk of osteosarcoma is seen in rats exposed to high doses. C onsequently, teriparatide is contraindicated in patients with risk of osteosarcoma, such as those with Paget disease, previous skeletal radiation, or unexplained elevation of alkaline phosphatase level. Teriparatide is approved for the treatment of postmenopausal women with severe bone loss, men with osteoporosis who have a high risk of fractures, and persons who have not improved on bisphosphonate therapy. O ne study suggests that it is advisable to follow teriparatide therapy with bisphosphonate therapy to maintain BMD gained[106].

Hormone therapy

The Women's Health Initiative confirmed that estrogen, with or without progesterone, slightly reduced the risk of hip and vertebral fractures, but found that this benefit did not outweigh the increased risk of stroke, venous thromboembolism, coronary heart disease, and breast cancer, even for women at high risk of fractures[107]. Lower

doses of conjugated equine estrogens and estradiol have been shown to improve BMD, but the reduced risk of fracture has not been demonstrated[108] and the safety is unknown. The FDA recommends hormone therapy for osteoporosis only in women with moderate or severe vasomotor symptoms, using the lowest effective dose for the shortest time.

Combination therapy

Bisphosphonates do not have additive effects on BMD when used concomitantly with parathyroid hormone[106], but they do have additive effects on BMD when combined with hormone therapy[108,109]. A ntifracture effectiveness of these combinations has not been shown. A lthough research continues, there is currently a limited role for combination therapy beyond subspecialty consultation or clinical trials.

Animal models of osteoporosis

Animal models provide more uniform experimental material and allow for extensive testing of potential therapies. A carefully chosen, appropriate experimental animal model for the study of osteoporosis minimizes the limitations associated with studying the disease in humans, namely time and behavioral variability among test subjects. Since 1994, the US Food and Drug Administration (FDA) requires data from both the rat and a well-validated large animal model for preclinical evaluation of new experimental drug therapies at a clinical dose and 5 times the dose. The high cost and long time frame of clinical testing are other reasons why animal models play a crucial role in osteoporosis research[110]. Even a model with a small representation of human functions may be of use for some aspect of the human condition under examination[111].

An additional goal for research into osteoporosis is the design of prosthetic devices (with or without biological coatings to promote osseointegration) that will perform optimally in the presence of osteoporotic bone. The biological and biomechanical characteristics of orthopaedic implants, bone-graft substitutes (with or without osteogenic bone morphogenetic proteins) can be tested on large numbers of animals maintained with a level of experimental control, impossible in human clinical research[112].

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[113]. 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[114]. 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[115,116] contrary to the impression given in the FDA guidelines. In addition, bone loss in aging occurs at endosteal surfaces adjacent to the marrow[117]. Even the cortical bone displays a low level of intracortical remodeling in the rat that is readily induced by stressful metabolic conditions[118,119]. The major drawback of the rat skeleton is that some bones retain lifelong growth and do not fuse epiphyses[120]. Many long bone epiphyseal growth plates in the male rat remain open past 30 months[120]. 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[121,122], 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 Im/d, femoral head of < 1Im/d[123] and the distal tibia epiphyseal growth plate is closed[120,124]. Periosteal expansion at long bone diaphysis continues until about 10 months, marking the age of peak bone mass[125,126] allowing ample time for experimental designs to prevent and restore bone mass and strength.

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[127,128]. The local immobilization or disuse model usually are performed in one limb. Other methods of disuse include nerve[129], spinal cord[130,131] or tendon resections[132], casting[133], bandaging of one limb[134] or suspension of both hindlimbs[135] 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. 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[136]. 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[137].

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 ovariectomized rat model and one larger bone remodeling species[113]. 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[138]. Bone turnover rates were increased for up to 2 years in ovariectomized monkeys as evidenced by increased serum and urine markers and increased bone formation rates measured histomorphometrically[139]. 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 dualenergy X-ray absorptiometry and decreased bone strength using biomechanical testing of the spine and femoral neck. Recently the detailed changes in the cortical bone of the humeral and tibial shaft in adult ovarectomized cynomolgus monkeys treated with one and 5 Ig PTH (LY333334)/kg/d for 18 months have been reported[140]. 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).

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[115]. Even the cortical bone proper in the rat displays low levels of intracortical remodeling and the prevailing activity at the endocortical surface are remodeling[116]. 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[140]. 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. 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. 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[141,142]. 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 osteoporosis1 has low peak bone mass and develops fractures in old age[143]. Bone development is normal during the first 3 months, but osteopenia progressively develops thereafter[144]. 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[145] 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. 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[146,147] have demonstrated that the mouse can be a reliable animal model of glucocorticoidinduced 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.

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